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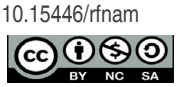

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EVALUADORES

El Comité Editorial dentro de sus políticas, envía los artículos a especialistas, con el fin de que sean revisados. Sus observaciones en adición a las que hacen los editores, contribuyen a la obtención de una publicación de reconocida calidad en el ámbito de las Ciencias Agrarias. Sus nombres son mencionados como una expresión de agradecimiento.

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EDITORIAL

El dolor de una partida: lamentando una ausencia

"Cuando alguien va al teatro, a un concierto o a una fiesta de cualquier índole que sea, si la fiesta es de su agrado, recuerda inmediatamente y lamenta que las personas que él quiere no se encuentren allí". Federico García Lorca, 1931.

Marcar los ritmos de los acontecimientos y sus protagonistas es tarea de la historia y factura de los historiadores. Hoy, sólo pretendemos rescatar la memoria y el legado del insigne Maestro Fabio Bustamante Betancur.

Comenzando por recordar que, en el marco de la segunda Conferencia Latino Americana sobre Educación Superior, celebrada en Medellín en 1962, se consolidó la inquietud de crear un programa de estudios para Ingeniería Agrícola. Fue en aquel momento cuando el señor Decano de la Facultad de Agronomía, (1957-1963), doctor Carlos Garcés Orejuela, designó al profesor Fabio Bustamante Betancur, profesor de Riegos y Drenaje de la mencionada Facultad, como encargado de coordinar las labores y hacer las gestiones necesarias para preparar el programa de estudios de la carrera de Ingeniería Agrícola, el cuál fue revisado y avalado previamente por las siguientes instituciones: Universidad Agraria de La Molina - Perú, Facultad de Minas de la Universidad Nacional de Colombia, Sede Medellín, Universidad Nacional de Colombia Sede Palmira, Universidad de Míchigan, Universidad de Nebraska, IICA (Instituto Interamericano de Ciencias Agrícolas), FAO (Organización de las Naciones Unidas para la Alimentación y la Agricultura), OEA (Organización de Estados Americanos). El plan de estudio de Ingeniería Agrícola preparado por el profesor Fabio Bustamante Betancur con las asesorías antes mencionadas, se presentó en 1964 a consideración del Consejo Superior de la Universidad Nacional de Colombia, quien lo hizo realidad en 1965 con la iniciación de estudios de 25 aspirantes, de los cuales egresaron 13 en 1970, para satisfacción plena del gestor del primer Plan Curricular de Ingeniería Agrícola en Colombia, organizado en la sede Medellín, producto de su dedicación y esfuerzo.

En ese norte, queremos hacer mención muy sentida al profesor Emérito Fabio Bustamante Betancur, en medio de los buenos recuerdos y compungidos por su partida hacia la eternidad, ya que su augusta figura no nos acompañará más, su inspiración y legado sí, y, de ello queremos dejar constancia, rindiendo este homenaje póstumo. Admitiendo que, no necesariamente, la longitud de las palabras, podrá dar cuenta de sus logros y de su sabiduría, puesta permanentemente a disposición de su querida Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia Sede Medellín, señalando el camino y despejando la mente de los que fuimos muy afortunados de haber compartido sus enseñanzas.

Con ese su sueño, hecho realidad desde 1965, se ha contribuido a influenciar los procesos de transformación de la bucólica sociedad rural-agraria colombiana, en una opción de vida y de desarrollo económico y social. Queda, en consecuencia, el compromiso de recibir la posta, esperando ponerla a buen recaudo para trascender lo construido por este visionario, quien le apostó a la modernización de la agricultura colombiana, en particular, con el manejo de los recursos de agua y suelo, a través de la implementación de los sistemas de riego y drenaje, una de sus especialidades, para ese tiempo bastante incipiente en Colombia.

Sus enseñanzas nos pondrán tono con los alcances de la futura Ingeniería Agrícola, donde sus ejecutarías se centralizarán en la obtención de desarrollos tecnológicos para la producción agrícola y agroindustrial, mediante

reconversiones de energía e implementación de la inteligencia artificial, bajo un manejo eficiente y sostenible. Llegó el momento de apropiar y desarrollar ese concepto lejano que constituye la agricultura inteligente, intensiva en microelectrónica, software especializado, bases de dados, etc. Todo lo cual, se puede traducir en una agricultura altamente productiva y amigable con el medio ambiente. Por ello, la agricultura de hoy, como ayer, debe ser la estrategia de adaptación ecológica al entorno y no un sistema cultural que busca el dominio de la naturaleza y el reinado del hombre supranatural.

En hora buena, y muy merecidamente el Laboratorio de Riegos y Drenaje de la Facultad de Ciencias Agrarias, que hoy lleva su nombre, será el atril y pebetero que dé luz a su nombre y avive el fuego de la pasión, por el trabajo bien hecho. Es decir, “habrá luz en la poterna y guardia en la heredad”.

En ese norte, a ese ser especial que iluminó las vidas de todos los que tuvimos la gran fortuna de concurrir a los diferentes cursos que impartió durante su permanencia en la universidad y a otras de sus actividades académicas, en donde nos señaló rutas y metas, eterna gratitud. Hoy ha pasado a la historia, en medio de un profundo dolor, dejando un espacio en la memoria colectiva de quienes tuvimos el privilegio de conocerlo y de compartirle. Entonces, cómo no recordar y enaltecer la memoria de este gran maestro, cuando, sin duda, su labor imprimió en la carrera de Ingeniería Agrícola un espíritu de humanismo excepcional y de profesionalismo integral, que sobrevivirá a través del tiempo.

En resumen, hoy, más que nunca, la Ingeniería Agrícola es necesaria como arsenal importante en la lucha contra el hambre y la pobreza para proteger el medio ambiente, garantizar la seguridad alimentaria, la salud humana y la de otros seres vivos, aportando a través de la ciencia y la tecnología en la transformación estructural del campo, contribuyendo en crear las condiciones de bienestar para la población rural y de esta manera contribuir a la construcción de una paz estable y duradera en nuestro país.

De eso estará orgulloso el profesor Emérito Fabio Bustamante Betancur, desde su celosa vigilancia. ¡No lo defraudaremos!

Comité Asesor de Carrera Ingeniería Agrícola
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Research article

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Influence of soil cover and herbicide application on weed control and corn yield

UNIVERSIDAD NACIONAL DE COLOMBIA

Influencia de la cobertura del suelo y la aplicación de herbicidas en el control de malezas y la productividad del maíz

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ABSTRACT

Crop management and herbicide rotation have influenced the sustainability of production systems. The cover crops use and pre-and post-emergence herbicides are important tools that help farmers' strategies and conserve the agricultural system. In this context, the objective of this research was to evaluate the dry matter production of different cover crops before the establishment of corn, the effect on decreasing weed population, and the increase in corn yield. In addition, the influence of pre-and post-emergence herbicides on summer cultivation, observing the behavior concerning weeds, crop injury, and crop yield. The experiment was conducted during the years 2018-2019 in Sertão/RS - Brazil. The experimental arrangement was of randomized blocks with four replications. The treatments used were three different winter cover crops preceding corn cultivation × four pre-emergence herbicides × four post-emergence herbicides, totaling 192 experimental units. Going through the results, atrazine and atrazine + simazine used in pre-emergence had more influence on weed number reduction, and the post-emergence ammonium glufosinate herbicide promoted the highest weed control in post-emergence. Amicarbazone and glyphosate resulted in the best combination for corn yield using in pre-and post-emergence, respectively. Rye + turnip + vetch as cover crop resulted in higher biomass production, more significant weed number reduction, and increase corn yield.

Keywords:
Avena strigosa L
Consortium
Secale cereale L
Zea mays L

RESUMEN

El manejo de cultivos y la rotación de herbicidas han influido en la sostenibilidad de los sistemas de producción. El uso de cultivos de cobertura y herbicidas de pre y post-emergencia son herramientas importantes que ayudan a los agricultores a desarrollar estrategias y conservar el sistema agrícola. En este contexto, el objetivo de esta investigación fue evaluar diferentes cultivos de cobertura previos al establecimiento del cultivo de maíz en relación con la producción de materia seca, el efecto en la disminución de la población de malezas y el aumento del rendimiento del maíz. Además, la influencia de los herbicidas de pre y postemergencia en el cultivo de verano, observándose el comportamiento en relación a malezas, daño al cultivo y rendimiento del cultivo. El experimento se realizó durante los años 2018-2019 en Sertão/RS - Brasil. El arreglo experimental fue de bloques al azar con cuatro repeticiones. Los tratamientos utilizados fueron tres diferentes cultivos de cobertura de invierno que preceden al cultivo de maíz × cuatro herbicidas de preemergencia × cuatro herbicidas de postemergencia, totalizando 192 unidades experimentales. Al analizar los resultados, la atrazina y la atrazina + simazina utilizadas en preemergencia tuvieron más influencia en la reducción del número de malezas, y el herbicida glufosinato de amônio promovió el mayor control de malezas en postemergencia. La amicarbazona y el glifosato resultaron en la mejor combinación para el rendimiento de maíz en pre y postemergencia, respectivamente. El centeno + nabo + arveja como cultivo de cobertura dieron como resultado una mayor producción de biomasa, una reducción más significativa del número de malezas y un aumento del rendimiento del maíz.

Palabras clave:
Avena strigosa L
Consorcio
Secale cereale L
Zea mays L

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No-till system is considered the most widely and sustainable practice for agricultural production in the Brazilian agroecosystems (Fuentes-Llanillo *et al.*, 2021). One of the premises of this management is the rotation of cash and cover crops, maintaining the soil constantly covered, using the alternation of different crops in the same area. Therefore, the same species return to the same location, following the interval occupied by other crops. In contrast, agricultural systems based on crop succession result in low biomass production that keeps the soil uncultivated during specific periods of the year, promoting degradation, the presence of problematic weeds species, leading the systems to be less efficient and unsustainable due to increased costs, yield stagnation, and the evolution of weed resistance species (Barbieri *et al.*, 2019; Adami *et al.*, 2020).

From this conceptualization, the sustainability of the farm system involves integrated management tools, in which cover crops preceding major crops, in isolated or in a consortium can bring numerous benefits to the production system (São Miguel *et al.*, 2018). The use of cover crop mixtures promoted a beneficial intraspecific competition to the system, making a physical barrier by the straw that reduces the amount and quality of light, the wavelength of waves, and the thermal amplitude that reaches the soil, which are the most stimulating environmental factors to overcome dormancy by weeds (Gomes and Christoffoleti, 2008). Additionally, there is a reduction in the stimulation of germination processes and in weed growth, which presents propagules with low seed reserve (Brighenti and Oliveira, 2011).

Complementary, the use of different chemical management strategies in the control of weed species, in which the rotation between mechanisms of action and the use of pre-emergent herbicides provide effective results and turns into an indispensable tool. This integrated management promotes efficient weed control, with the aim of controlling populations at the beginning of their development and exploring the residual herbicide effect in order to keep the crop out of competition during the critical period of infestation, ensuring raising success in productivity (Galon *et al.,* 2018). Also, previous research has found that the weed control stage to reduce greater than 5% yield loss in corn, must be before 11 cm height and before 27 days after corn emergence, highlighting the relevance of effective early weed control (Soltani *et al.*, 2022). Thus, the need for integrated tools for the management of agricultural production systems justifies the realization of related studies, which can help professionals and farmers increase adaptation for sustainability and profitability work. In this context, this study aimed to evaluate the influence of different cover crops in dry mass accumulation, its impacts on weed population, and the effects on corn crop yield. Besides, the use of different pre-and post-emergent herbicides on corn, to evaluate weed control, crop selectivity, and crop yield.

MATERIALS AND METHODS

General description. The experiment was performed in Sertão/RS - Brazil (28°03′18″ S and 52°14′53″ W), at 670 masl. The climate of the region according to the Köppen classification is "Cfa", with 17.8 °C average annual temperature and 1.791 mm of average annual rainfall. The soil of the site is classified as Deep Dystrophic Red Nitossol, with 49% of clay and 2.2% of organic matter, according to soil collection and analyses shown in Table 1.

Table 1. Chemical and physical soil analysis. Sertão/RS, 2020.

H_2O	mg dm^{-3}		cmolc dm^{-3}					% (M/V)	
pH	P	K	Al	Ca	Mg	H+Al	CEC (pH 7.0)	OM	Clay
5.6	21.1	26.5	0	5.74	2.35	3.35	12.64	2.2	49

Experimental design. The experimental arrangement was randomized blocks with four replications, using three winter covers × four pre-emergence herbicides × four post-emergence herbicides in corn, totaling 192 experimental plots. The cover crop treatments used during the winter were fallow; black oats (*Avena strigosa* L.) with a density of 350 plants m^{-2} and; crop mixure using rye (*Secale cereale* L.) + vetch (*Vicia sativa* L.) + turnip (*Raphanus sativus* L.) in 210 plants m^{-2} of rye + 60 plants m^{-2} of vetch + 50 plants m^{-2} turnip that means 60% - 20% - 20% of

the recommended number of plants from each species, respectively. These cover crop species were chosen based on regional adoption by farmers and their strong performance in cover crop use.

Each experimental plot was 5 m long and 3.5 m wide, with seven corn rows spaced 0.45 m between them. Within each unit, the evaluated area was organized into plots with an area of 5.4 m², 4 m in length, and three central rows of corn, to remove possible border effects. The cover crop treatments were sown with a seeding/fertilizer (Semeato® 15/17), with 17 seed lines spaced 0.17 m between them. For corn sown, was used the sower/fertilizer Kuhn® PG PLUS 700, with seven seed lines spaced 0.45 m between them. The corn hybrid used was the Pioneer® P3565PWU, with Agrisure Viptera, Powercore Ultra, Herculex 1, Liberty Link®, and Roundup Ready® 2, with a final population of 7 plants m^{-2}. Fertilization was performed according to the recommendations of the Brazilian Society of Soil (2004).

The pre-emergent herbicides used on corn were non treated; atrazine (2.5 kg a.i. ha^{-1}); atrazine + simazine (1.625 + 1.625 kg a.i. ha^{-1}) and; amicarbazone (0.14 kg a.i. ha^{-1}). The post-emergent herbicides on corn were non treated; glyphosate (1.92 kg a.i. ha^{-1}); ammonium glufosinate (0.4 kg a.i. ha^{-1}) and; nicosulfuron (0.08 kg a.i. ha^{-1}).

Sample collection and evaluations during cover crop period. The dry mass of the cover crops was determined within a square of 0.5 × 0.5 m. These samples were collected in each plot 30 days before corn was planted and harvested at the soil level. The weed number was counted on the same day. The cover crop samples were placed in an oven at 65 °C until a constant mass was obtained. The dry mass for each plot was weighed and the weed number was used for the statistical analysis in each cover crop treatment.

To eradicate the cover crop, glyphosate (1.92 kg a.i. ha^{-1}) was sprayed and 12 days after the first application, paraquat (0.4 kg a.i. ha^{-1}) was used. The application of all herbicide treatments was performed with a backpack sprayer pressurized by CO_2, using the spraying nozzle model TeeJet XR110015 spaced 0.5 m between them, at 3.0 bar, at a constant velocity, and a volumetric flow of 180 L ha^{-1}.

Corn crop details. The amicarbazone treatment was applied on September 30th, 2019, 11 days before corn was sown, according to the recommendations. Right after sown was planted on October 11th, 2019, the others pre-emergence treatments were sprayed. Post-emergence treatments were sprayed when the crop was between V_3-V_4 vegetative state, according to herbicide recommendation.

Weed number and corn injuries rates were collected at 7 and 14 days after crop emergence (DAE), which means 14 and 21 days after herbicide application (DAA). The visual control effect on weeds and the injuries on corn from post-emergent herbicides were performed at 7, 14, 21, 28, and 35 DAA. To evaluate these injuries and weed control, the percentage scores were used, being zero the absence of weed control or injuries in crop and a score of 100 means the total weed control or complete death of corn plants. All crop management was performed as needed during the life cycle, expecting higher yields. The corn was harvested manually; grains were weight and then moisture was determined. Results were expressed in $kg.ha^{-1}$.

Statistical analysis. The data obtained were verified regarding the homogeneity of variance and subsequently submitted to variance analysis ($P \leq 0.05$), using the software ASSISTAT 7.7 BETA (Silva and Azevedo, 2016). A significant effect was verified by the evaluated parameters, and the means were compared using the Scott-Knott cluster test ($P \leq 0.05$) for dry mass production, the number of weeds in the winter period, and corn yield. For weed number during the corn season and herbicides injury Tukey's test was used ($P \leq 0.05$).

RESULTS AND DISCUSSION

Dry matter and weeds in the winter period. Regarding the dry mass in the fallow, the production was mainly originated from weeds that emerged during the winter, predominantly ryegrass *(Lolium multiflorum* L.) (Table 2). This treatment showed high variation in density and weed flow, producing low dry mass, even when samples with a high number of plants m^{-2} were collected, indicating a higher variation. In some plots, more than 100 plants m^{-2} were counted, demonstrating the high germination rate of this species that are widely present in the seed bank mainly in southern Brazil.

The predominance of ryegrass is related to its highly competitive ability, adaptation, easy dispersal, natural

dryness, and few control alternatives due to resistance to EPSPS, ALS, and ACCase herbicides, making it a troublesome and also increasing herbicide costs with above 57% (Tironi *et al.,* 2014; Vargas *et al.,* 2015). When managed properly, ryegrass can be a crop favorable to corn in its succession. However, when it is a weed, it can produce less dry mass, compromising the development and productivity of corn through competition and the release of allelochemical compounds (Franz *et al*., 2020; Moraes *et al.,* 2013).

Several other weed species at different growth stages were observed in the area without crop in winter. This scenario makes weed management more challenging, and also the lack of crops inadequately protects the soil during this season. In the treatments with oats and the crop mixture, the implementation with seeders ensures uniform plant growth above the soil and increases mass production, which improves the sustainability of the system and the easy use of herbicides, reducing the infestation of weeds and improving the yield of subsequent crops (Martins *et al.,* 2016).

The establishment of oats and the crop mixture was carried out by the sower, which stimulated the growth of weed species in the sowing line, where soil tillage took place. Nevertheless, no significant subsequent flows were observed, possibly due to the rapid closing of the space between rows and homogeneous coverage of the area, presenting a number of weeds inversely proportional to the speed with which the vegetation cover could cover the ground and the ability to increase above ground mass production. Likewise, it was observed that the dry mass production capacity in intercropping was significantly higher, which is in agreement with previous research that concluded that farmers can potentially add the intercropping pattern to their crop systems to maximize the functions provided by cover crops (Bybee-Finley *et al*., 2022).

Table 2. Dry mass production and weed number at each cover crop treatment during winter season. SERTÃO/RS, 2020.

Treatment	Dry matter (ha^{-1})	Weeds (m^2)
Fallow	880.0 c*	15.1 a
Oats	2754.9 b	10.4 b
Rye + turnip + vetch	3226.7 a	5.8 c
Average	2287.2	10.4
Variation coefficient	13.6%	22.1%

* Means followed by different letters in the columns indicate significant differences by the Scott-Knott test ($P \leq 0.05$).

Weed control. It is known that the soil dynamics of pre-emergent herbicides are highly dependent on the edaphoclimatic conditions to which they are subjected. Precipitation of about 20 mm is critical for the herbicide to transpose the dry mass layer and be active in soil solution (Maciel and Velini, 2005). Moreover, there is a positive correlation between rainfall and the leaching of herbicide molecules, which may compromise the herbicide's effect on target plants when they are in high volume (Monquero *et al*., 2008). Corn sowing and pre-emergence spraying were performed on the same day during October, with elevated rainfall and temperatures that year (Figure 1). These climatic conditions were favorable for the rapid emergence of crops and weeds. The weed number during the corn crop is provided in Table 3.

Regarding the winter cover crop treatments, at 7 DAE, or 14 DAA, the emerged weed number was higher in the presence of cover crop, compared to the fallow. It is assumed that the absence of crop remains and the moisture in the soil allowed a faster action of the herbicide in the soil solution since the aerial mass continues to be a physical barrier for almost pre-emergent herbicides (Matos *et al.,* 2016). However, after the herbicide reaches the soil, usually by rain, the remaining crop layer promotes further distribution and persistence of the herbicide in the soil, due to the channels formed between remaining plants and soil microorganisms and, mainly, by the protection of degradation processes to which the herbicide molecule is vulnerable when it is outside of crop residues (Sorenson *et al.,* 1991).

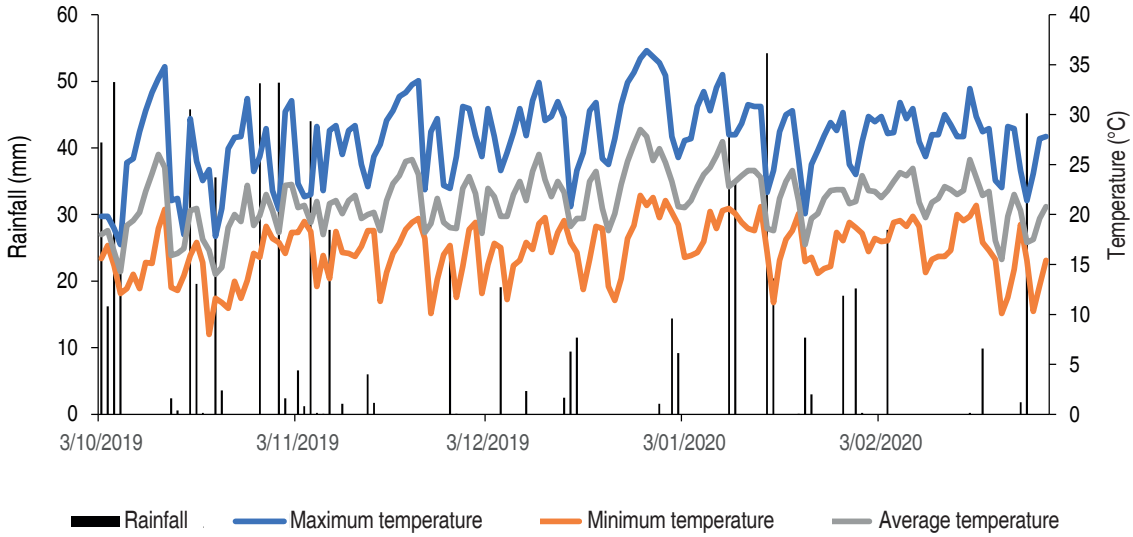

Figure 1. Precipitation, average, maximum and minimum temperature during corn season in SERTÃO/RS, 2020.

Table 3. Number of weed plants per m² after pre-emergence herbicide sprayed. SERTÃO/RS, 2020.

Emerged weeds 14 days after sprayed		Emerged weeds 21 days after sprayed	
Average weed number between botanical class			
Monocots	14.8 b*	Monocots	16.2 b
Dicots	10.9 a	Dicots	12.5 a
Average weed number between different cover crop			
Oats	14.2 b	Oats	15.9 a
Fallow	11.5 a	Fallow	13.1 a
Rye + Turnip + Vetch	13.0 ab	Rye + Turnip + Vetch	14.2 a
Average weed number between different pre-emergent herbicides			
Atrazine	3.5 a	Atrazine	4.5 a
Atrazine + Simazine	3.1 a	Atrazine + Simazine	4.0 a
Amicarbazone	6.0 a	Amicarbazone	9.9 b
Untreated	38.9 b	Untreated	39.2 c

* Means followed by same letters in the columns indicate no significant differences by Tukey's test ($P \leq 0.05$).

In the subsequent evaluation (21 DAA), it was observed that all winter treatments were similar when related to weed number. In relation to the herbicides used in corn post-emergence, atrazine and atrazine + simazine treatments achieved great control of weeds (Table 4). Previous research showed that the interception of the herbicide amicarbazone by straw, and its transposition before precipitation occurs, may suffer several modifications (Cavenaghi *et al.*, 2007). The weed number from the non-herbicide sprayed treatment indicates the relevance of pre-emergent herbicides management in corn, regardless of the cover crop presence before, since, from 11 DAE, the crop already is affected by weed interference (Galon *et al.*, 2008).

No crop injuries were observed (data not provided), which demonstrates the selectivity of these herbicides, which supports their widespread use during corn cultivation. The

ammonium glufosinate had the best performance on weed control at 7 DAA, presenting an average control of around 80%. Rapid chlorosis of the treated tissue, followed by necrosis and death of plants after a few days caused by the herbicide actions on the target plants, was responsible for the high level of damage observed (Brunharo *et al.,* 2014). After 21 DAA, the growth of plants that were in the initial stage of development and were shaded by taller plants that received the herbicide application, resulted in a drastic decrease in injuries according to visual evaluations, justifying the non-presentation of these additional data, once these weeds did not show injuries and the visual data was approximately 0% of phytotoxicity.

In relation to post-emergence herbicides treatments, glyphosate promoted fewer symptoms in weeds in the first evaluation at 7 DAA, which may result from its action mode demonstrating slow control, allied to the presence of resistance weeds species, events that have become widespread after Round Ready® technology (Brunharo *et al.,* 2014; Heap and Duke, 2018). Nonetheless, the herbicide effects were longer-lasting, presenting injuries in weeds until the evaluation of 21 DAA.

Intercropping pattern improves weed control. It seems a good alternative since besides enabling a higher biomass production and providing benefits related to soil conservation, helps in moisture maintenance and nutrient cycling and also decreases weed number. It does this by increasing herbicides efficacy and biological decomposition activity, as well as the decomposition of weed seeds present in the soil bank, and by providing a physical barrier that prevents the stimulation of emergence, especially in weed species with positive photoblastic characteristics (Chu *et al*., 2017; Chahal and Van Eerd, 2018; Ottavini *et al*., 2019).

Table 4. Visual weed control using post-emergence herbicide on corn. SERTÃO/RS, 2020.

7 days after application		14 days after application		21 days after application	
Average weed number between botanical class					
Monocots	54.2 a	Monocots	5.0 a	Monocots	2.3 a
Dicots	53.5 a	Dicots	5.0 a	Dicots	2.3 a
Average weed number between different cover crop					
Oats	48.8 b	Oats	7.3 a	Oats	33.0 a
Fallow	47.2 b	Fallow	5.3 b	Fallow	3.0 a
Consortium	65.6 a	Consortium	2.5 c	Consortium	0.7 b
Average weed number between different pre-emergent herbicides					
Glyphosate	52.2 c	Glyphosate	9.1 a	Glyphosate	5.6 a
Glufosinate	80.0 a	Glufosinate	4.6 b	Glufosinate	1.3 b
Nicosulfuron	68.9 b	Nicosulfuron	4.8 b	Nicosulfuron	2.7 ab
Untreated	0.0 d	Untreated	0.0 c	Untreated	0.0 b

* Means followed by same letters in the columns indicate no significant differences according to Tukey's test ($P \leq 0.05$).

Grain yield. Cover crop use before cash crop had positive influence on grain yield. The higher productivity was obtained after intercrop use, followed by oats, presenting 12900 kg ha^{-1} and 9350 kg ha^{-1}, respectively (Table 5). The fallow had a reduction of more than 50% in corn productivity in relation to the crop consortium. These results are related to the organic material added to the system by the winter crop, which influences the dynamics of soil moisture and its use by the crop; in addition to promoting a mechanical and thermal barrier to the soil, providing better moisture conservation and reducing evapotranspiration losses concerning the uncovered soil (Gava *et al.,* 2013; Klein and Klein, 2015; Barbieri *et al.,* 2020).

Regarding the pre-and post-emergent herbicides used and the interaction with the corn yield, the amicarbazone

and glyphosate treatments resulted in higher grain yields, respectively. Satisfactory results in weed control have been found with the use of the herbicide amicarbazone by Ferreira *et al*., (2020). However, the use of this herbicide in relation to its residual and carryover potential in succession crops should be considered, depending on the used rate (Alonso *et al*., 2011). The performance of glyphosate demonstrates that despite its reduced efficiency in current weed populations, when it is used following correctly vegetative stage and rate, it demonstrated satisfactory results in weed control.

Table 5. Corn yield. SERTÃO/RS, 2020.

	Corn yield (kg ha^{-1})
Average between winter cover crops	
Oats	9.400 b
Fallow	5.904 c
Consortium	12.841 a
Average between pre-emergent herbicides	
Atrazine	7.966 c
Atrazine + Simazine	10.221 b
Amicarbazone	11.778 a
Untreated	6.561 d
Average between post-emergent herbicides	
Glyphosate	9.996 a
Glufosinate	9.405 c
Nicosulfuron	9.833 b
Untreated	8.292 d

* Means followed by same letters in the columns indicate no significant differences according to the Scott-knott test ($P \leq 0.05$).

CONCLUSIONS

The intercropping pattern promoted higher biomass accumulation, decreased weed number, and improved corn productivity. Atrazine and atrazine + simazine treatments had the greatest reduction in the weed number used by pre-emergence herbicide, and the ammonium glufosinate provided the highest levels of control in post-emergence corn. The treatments amicarbazone and glyphosate obtained the best performances in relation to crop yield when used during pre-and post-emergence, respectively.

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Postharvest behavior and chilling injury in avocado (*Persea americana* Mill) fruit cv. Hass treated with 1-methylcyclopropene, ethylene, and intermittent warming

Comportamiento poscosecha y daños por frío en frutos de aguacate (*Persea americana* Mill) cv. Hass tratado con 1-metilciclopropeno, etileno y calentamiento intermitente

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ABSTRACT

Keywords:
Chilling injury
Postharvest quality
Ripening
Shelf-life

Persea americana, cv. Hass has become an important crop in Colombia due to increased exports to European markets; however, the avocado is a climacteric fruit sensitive to chilling injury, reducing its shelf-life and affecting commercialization abroad. This research aimed to evaluate the effect of 1-methylcyclopropene, ethylene, and intermittent warming on chilling injury and postharvest behavior of avocado cv. Hass. Five treatments were evaluated: Control; Intermittent warming cycles of 4 days at 2 °C + 1 day at 18 °C; Intermittent warming cycles of 7 days at 2 °C + 1 day at 18 °C; 1-methylcyclopropene, and ethylene. The fruits were stored for 29 days at 2 °C and then up to 14 days in the shelf-life period. The intermittent warming treatment 4-days cycles reduced chilling injuries. However, this treatment increased weight loss, respiration, epidermal color index, and electrolyte leakage during the storage period at 2 °C. Intermittent warming showed that Hass avocado fruit ripens faster, regardless of the application of ethylene in the shelf-life period. The results indicated that intermittent warming reduced the chilling injury, the treatment with 1-methylcyclopropene delayed ripening but it is not recommended for avocado cv. Hass when stored at 2 °C because the chilling injury is not reduced.

RESUMEN

Palabras clave:
Daño por frío
Calidad poscosecha
Maduración
Vida útil

Persea americana, cv. Hass se ha convertido en un cultivo importante en Colombia debido al aumento de las exportaciones a los mercados europeos; sin embargo, el aguacate es una fruta climatérica sensible al daño por frío, que tiende a reducir su vida útil, afectando la comercialización en el exterior. Esta investigación tuvo como objetivo evaluar el efecto del 1-methylcyclopropene, el etileno y el calentamiento intermitente sobre el daño por frío y el comportamiento poscosecha del aguacate cv. Hass. Se evaluaron cinco tratamientos: Control; Calentamiento intermitente: ciclos de 4 dias a 2 °C + 1 dia a 18 °C; Calentamiento intermitente: ciclos de 7 dias a 2 °C + 1 día a 18 °C; 1-methylcyclopropeno, y etileno. Los frutos se almacenaron durante 29 días a 2 °C y luego hasta 14 días en el período de vida útil. El tratamiento de calentamiento intermitente con ciclos de 4 días redujo los daños por frío. Sin embargo, este tratamiento aumentó la pérdida de peso, la respiración, el índice de color de la epidermis y la fuga de electrolitos durante el período de almacenamiento a 2 °C. El calentamiento intermitente generó una maduración más rápida del fruto de aguacate Hass, independientemente de la aplicación de etileno en el período de vida útil. Los resultados indicaron que el calentamiento intermitente redujo el daño por frío, el tratamiento con 1-methylcyclopropene retrasó la maduración pero no se recomienda para el aguacate cv. Hass almacenado a 2 °C porque no se reduce el daño por frío.

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The avocado (*Persea americana* Mill) cv. Hass is a species in the Lauraceae family and has significant nutritional importance because of its high content of proteins, vitamins, and minerals, which have a beneficial effect on health (Bhuyan *et al*., 2019). Colombia is among the top eight world exporters of fresh avocado, with a promising socio-economic future (Arias and Moors, 2018; Ramírez-Gil *et al*., 2019).

Postharvest losses of perishable products worldwide can reach 60%, which is higher in developing countries (Yahida, 2020). Therefore, improved storage and preservation techniques are needed for these products, especially cold storage in avocados (Yahia and Woolf, 2011). Despite the open market in Colombia for exporting avocado cv. Hass (Ramírez-Gil *et al*., 2019), there are significant limitations in terms of storage since prolonged periods of storage at low temperatures can cause chilling injury by altering the stability of cell membranes and changing the lipid phase (Pesis *et al*., 2002; Woolf *et al*., 2003). The recommended storage temperatures for avocado are between 6 and 8 °C for 2 or 4 weeks (Woolf *et al*., 2003). Chilling injury symptoms can occur after 4 weeks of cold storage at about 6 °C, depending on different factors such as maturity stage and growing conditions (Munhuweyi *et al*., 2020). On the peel, skin pitting, scalding, water-soaked appearance, failure to ripen, blackening, off-flavor, and decay are symptoms identified (Yahia and Woolf, 2011; Munhuweyi *et al*., 2020). In the pulp, chilling injury symptoms are characterized by flesh browning and increased susceptibility to the pathogen (Yahia and Woolf, 2011).

Because of its high respiratory rate and high production of ethylene, which are typical in climacteric fruit, the avocado is highly perishable and not very tolerant to long periods of transport and storage. Therefore, it requires special postharvest handling to maintain its organoleptic quality, such as applications of maturity retardants (Arias and Moors, 2018). Applications of 1-methylcyclopropene (1-MCP) reduce the process of maturation that leads to tissue softening, disintegration of the cell wall, and degradation of pigments. This maturity retardant is one of the more efficient ones because it occupies cell receptors, blocking the signal transduction cascade that leads to the expression of genes related to responses to ethylene, but it also inhibits the synthesis of ethylene (Balaguera-López *et al*., 2021). The chilling injury severity was affected by a 1-MCP treatment (1 µL L^{-1}) and stored in low oxygen in "Becon" avocados (Lurie and Pedreschi, 2014). Likewise, 1-MCP treatments have alleviated chilling injury in nectarine fruit through a reduction in the regulation of ROS and energy metabolism (Liu *et al*., 2018; Zhang *et al*., 2020). On the other hand, exogenous ethylene treatments have inhibited chilling injury by increasing proline and antioxidize activity content in pears (Wei *et al*., 2019).

To increase postharvest life and reduce losses, several studies recommend lowering the storage temperature, even around 2 °C, as was reported in avocado (Sivankalyani *et al*., 2015) and citrus fruits (Ghasemnezhad *et al*., 2008; Balaguera-López *et al*., 2019), however, this temperature causes chilling injury in several fruits of tropical and subtropical origin. Since the avocado is sensitive to chilling injury, heat treatments, such as intermittent warming, may be recommended for restorative action in fruit metabolism. Intermittent warming is a physically effective technique for decreasing chilling injury in many fruits (Liu *et al*., 2015). Intermittent warming consists of subjecting fruit to periodic rises in temperature during conventional cold storage and during the latency phase of alteration, with variable durations and intensity depending on the product. This treatment has been considered the most effective in minimizing chilling injury to plant organs (Sivankalyani *et al*., 2015), probably because of its ability to restore cell membranes altered by the cold, eliminating possible toxic metabolites accumulated in the cells, and tissue at low temperatures, or even favors the synthesis of some essential metabolites for cells (Heyes, 2018).

Affordable postharvest technologies are needed for producers with domestic and international production and marketing platforms that increase the useful life and reduce physiological damage in avocado fruit during storage. Therefore, the objective of this study was to evaluate the effect of 1-methylcyclopropene, ethylene, and intermittent warming on chilling injury and postharvest behavior of avocado fruit (*Persea americana* Mill) cv. Hass.

MATERIALS AND METHODS

Plant material, treatments, and storage conditions

The avocado fruits cv. Hass were at harvest maturity, in category 1 established by Colombian technical standard NTC 1248 and free of phytosanitary problems

and mechanical damage. They were collected from a commercial crop in the village of "La Costa" in the municipality of Soata, Boyacá, located at 6°19′58″ N, 72°41′02″ W at 1900 masl with an average annual temperature of 20 °C. This study was carried out in the Plant Physiology laboratory of the Faculty of Agricultural Sciences of the Pedagogical and Technological University of Colombia, Tunja, located at 5 °32′25″ N, 73°21′41″ W at 2787 masl. The room temperature was 18 °C, and the relative humidity in the laboratory was 65%.

A completely randomized design was used, and the following thermal treatments were evaluated: T1: Control fruit (without treatment), T2: fruit subjected to intermittent warming with cycles of 4 days at 2 °C + 1 day at 18 °C (IW-4 d), T3: fruit subjected to intermittent warming with cycles of 7 days at 2 °C + 1 day at 18 °C (IW-7 d), T4: application of 1-MCP (0.035 µL L^{-1}) and T5: application of ethylene (1000 µL L^{-1}). Each treatment was repeated four times, for a total of 20 experiment units, and each experimental unit consisted of approximately 2 kg of fruit.

The fruits were disinfected using a 5% sodium hypochlorite solution and allowed to dry for 30 min. Then, the fruits with the 1-MCP treatments were immersed in a 0.014% EthylBloc and Ethylene solution. The immersion for the two treatments was 10 min in a 48% ethephon solution as an ethylene source (González *et al*. 2021); the fruits were allowed to dry and stored in refrigerators at 2 ± 0.5 °C without packaging for 29 days.

In the refrigeration period, the intermittent warming treatments were carried out according to the established cycles (4 and 7 days). After 29 days, the refrigeration period was suspended, and the fruits were left at room temperature (18 °C) to evaluate the shelf-life period.

Determination of weight loss, color, and respiratory rate

Non-destructive parameters, such as weight loss, color, and respiratory rate, were evaluated every 3 days. For the destructive parameters such as total soluble solids, total titratable acidity, and firmness, three readings were taken during the experiment. Table 1 shows the initial values of the destructive and non-destructive parameters in the avocado "Hass" before the storage period. The chilling injury was evaluated by the loss of total electrolytes at the beginning and end of the shelf-life and estimated by the chilling injury index.

Table 1. Physicochemical parameters of "Hass" avocado fruits at the beginning of storage (n=5).

Parameter	Value
Firmness (N)	103.06
Total soluble solids (%)	2.94
Total titratable acidity (%)	0.086
Color index of pulp	-1.25
Color index of skin	-13.73
Respiration Rate (mg CO_2 kg^{-1} h^{-1})	9.02

For the respiration rate, the fruits were placed in a 2 L hermetic chamber for 5 min, where the CO_2 emission product of respiration was determined by infrared CO_2 sensors coupled to a data capture system called Labquest. The weight of the samples was recorded to finally express respiration in mg CO_2 kg^{-1} h^{-1}. For weight loss (WL, %), a sample of approximately 100 g of fruit was measured for fresh mass, and, subsequently, Eq (1) was used:

$$WL(\%) = \frac{(W1 - W2)}{W1} \times 100 \qquad (1)$$

Where: W1=Initial fruit weight, and W2=Final fruit weight.

For the color of the epidermis and pulp, the CIELab system parameters "L*", "a*" and "b*" were determined at three equidistant points on the fruit using a CR-20 digital colorimeter (Konica Minolta). From these parameters, the color index (CI) was calculated by Eq (2) (González *et al*., 2021):

$$CI = \frac{1000 \times a^*}{L^* \times b^*} \qquad (2)$$

Evaluation of chilling injury index and electrolyte leakage

The chilling injury was evaluated at the end of the shelf-life using the chilling injury index (CII) according to Balaguera-López *et al*. (2019). The fruits were classified into different classes using a visual scale, and the following values were used: 0= no injury; 1= slight injury,

up to 10% damaged surface; 2= medium injury, between 10 and 50% of the surface with spots and 3= severe injury, more than 50% of the surface affected with larger damages, Eq (3) was used:

$$CII = \frac{\sum(\text{number of fruit in each class} \times \text{class value})}{\text{total number of examined fruit}} \quad (3)$$

For electrolyte leakage (EL, %), 10 cylinders were extracted from the fruit peel, 0.5 cm in diameter, with opposing orientations in each fruit sample. The pieces were placed in a test tube, and 30 mL of deionized water was added. The test tubes were vortexed for 1 min. The initial electrical conductivity (ECi) was measured by a digital conductivity meter (HI 9835 Hanna® - ICT, SL, range 0-29.9 µS cm^{-1}, precision±0.05 µS cm^{-1}). Subsequently, the tubes were incubated in a water bath for 15 min at 100 °C to extract all released electrolytes, then the final electrical conductivity (ECf) was measured. The electric conductivity values were expressed as a percentage of the highest value following Eq (4).

$$EL(\%)=(ECi / ECf) \times 100 \quad (4)$$

Determination of firmness, total acidity, and total soluble solids

For firmness (N), two measurements were taken in the equatorial zone of the fruit using a digital penetrometer (PCE-PTR200, range 0-200 N, precision±0.5 %) with a 6 mm strut. The total acidity (TA, % malic acid) was quantified using an acid-base titration applying NaOH (0.1 N) by a digital burette, using 5 g of pulp and phenolphthalein as indicator. The total soluble solids (TSS, %) were recorded with a Hanna brand digital refractometer with a range of 0 to 85% and a precision of 0.1%

Statistical analysis

The data analysis was performed by SPSS version 19. The tests of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were determined after the previous assumptions; an analysis of variance was carried out with a subsequent Tukey's multiple range comparison test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Cold storage

The avocado fruit weight loss increased with storage time and warming treatments ($P<0.05$). In general, there were no differences in weight loss between the control and the 1-MCP and ethylene treatments for each day of evaluation up to 5% loss on day 29. Intermittent warming is widely used to maintain the quality of climacteric fruit as Xi *et al*. (2012) reported for peaches (*Prunus persica*). In this study, the avocado fruit showed higher weight loss in the IW-4 d and IW-7 d treatments, 11 and 9%, respectively (Figure 1A). Mandarin (*Citrus reticulata*) fruit with IW treatments generated greater weight loss, associated with higher transpiration rates and higher respiratory rates (Balaguera-López and Palacios, 2018). In the case of the fruit treated with 1-MCP, the loss was expected to be lower because this compound interacts with ethylene receptors, blocking ethylene-dependent responses that occur during ripening (Yudou *et al*., 2019). The opposite occurred in this study, where the 1-MCP treatment did not significantly affect weight loss, possibly because the reduction in storage temperature declined the retardant's effectiveness (Blankenship and Dole, 2003).

The respiration rate decreased at the beginning of cold storage and then remained low during storage in all fruits, except those with intermittent warming because low temperatures affect enzyme kinetics (Ho *et al*., 2020). It has been reported that refrigeration is used to decrease the respiratory rate, preserve fruit quality, and delay senescence since, this decrease in respiration reduces the metabolic and enzymatic activity of fruit (González *et al*., 2021). In the IW-4 d treatment, the avocado fruit produced a high respiration rate over 23 days of storage. Similarly, the IW-7 d avocado fruit increased the respiration rate over 17 and 26 storage days (Figure 1B). These increases are due to changes in room temperature, as it has been reported for mandarins using intermittent warming (Balaguera-López and Palacios, 2018). Intermittent warming increases the respiration rate and free energy of water molecules, which increases their movement and exchange potential.

Ethylene has a close relationship with the respiratory rate of fruit. Therefore, 1-MCP applications are effective in reducing the respiratory rate of different fruit, as reported for tomato and avocado (Choi and Huber, 2008). Nevertheless, in this study on avocado fruit with storage at 2 °C, there was no effect of 1-MCP or ethylene on the respiratory rate, possibly because of the low temperature.

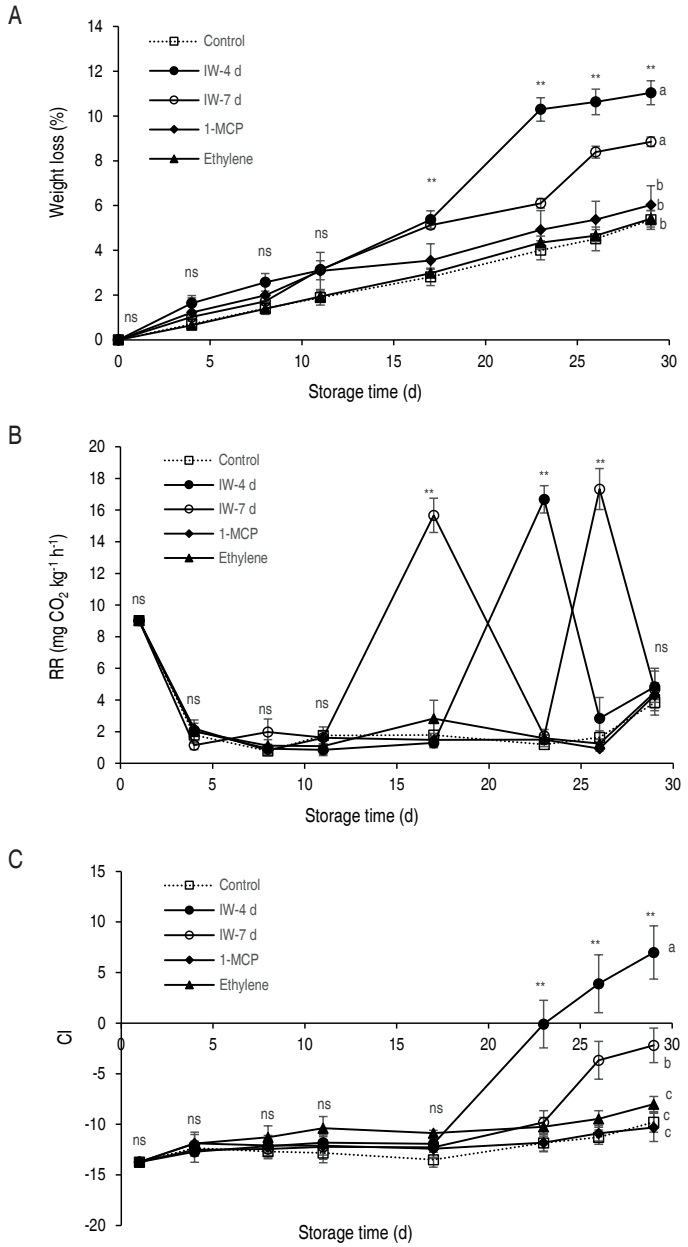

Figure 1. Effect of Intermittent Warming: IM-4 and IM-7; 1-MCP and Ethylene treatments on weight loss (A), respiration rate (mg CO_2 kg^{-1} h^{-1}) (B), and color index (CI) of epidermis (C) of "Hass" avocado fruits in storage at 2 °C. The vertical bars on each mean indicate the standard error (n=4). According to the analysis of variance, ns: no statistical differences, * differences at 5%, and ** 1%. Means followed by the same letter indicate no significant differences according to Tukey's test (P<0.05).

The color index (CI) in the epidermis showed statistical differences only after 23, 26, and 29 storage days of storage. The highest CI was obtained in the treatments IW-4 d (5.96) and IW-7 d (-2.19). The results of the intermittent warming agree with previous reports where changes in temperature resulting from interruptions in refrigeration normalized metabolic activity, chlorophyll degradation, and synthesis of anthocyanins as an indicator of ripening in avocado Hass fruit (Corrales-García *et al*., 2019). The CI of the epidermis of avocado fruit in the Control, 1-MCP,

and Ethylene treatments did not change significantly (Figure 1C). Cold storage at 2 °C efficiently reduced the metabolic activity related to color change in avocados, to the point that there is no effect of ethylene or 1-MCP. However, some studies have shown that 1-MCP decreases chlorophyllase activity and decreases chlorophyll losses (Sun *et al*., 2012). On the other hand, the pulp CI in the ethylene treatment had the highest value (-0.8), with the lowest value in the 1-MCP treatment (-1.9; Figure 2A). Lower values may be associated with a lower loss of chlorophyll as a result of a reduction in chlorophyllase activity as a result of 1-MCP treatments (Sun *et al*., 2012). Otherwise, ethylene has been reported to accelerate the degradation of chlorophyll and to be involved in the expression of pigments-encoding genes during ripening (Rodrigo and Zacarias, 2007).

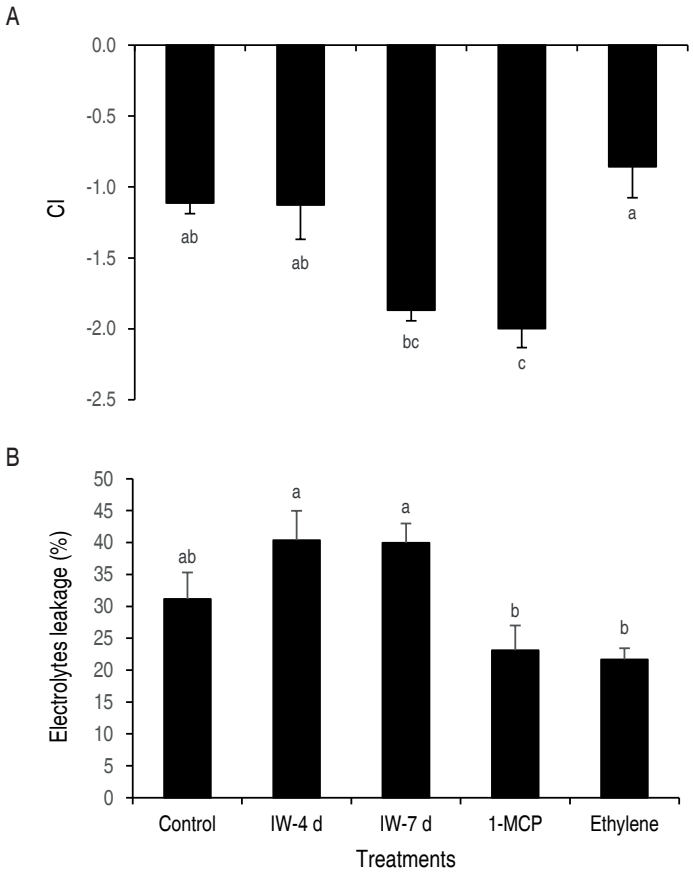

Figure 2. Effect of Intermittent Warming: IM-4 d and IW-7 d; 1-MCP and Ethylene treatments on the color index (CI) of epidermis (A), and electrolytes leakage (B) of "Hass" avocado fruits in storage at 2 °C. The vertical bars on each mean indicate the standard error (n=4). Means followed by the same letter indicate no significant differences according to Tukey's test (P<0.05).

Cell membrane stability, measured as the percentage of electrolyte leakage (EL), at the end of storage in the epidermis tissue of avocado fruit is shown in Figure 2B. The EL was lower in the 1-MCP (22%) and Ethylene (20%) treatments. Throughout maturation and senescence and under stress, a series of oxidative metabolic processes take place that affects the structure of cell membranes, altering their permeability and causing cellular dysfunctions, including the uncontrolled flow of electrolytes (Dai *et al*., 2021). Otherwise, the fruit with the greatest EL was subjected to IW-4 d (47%) and IW-7 d (48%; Figure 2B). Studies on mandarin and peach fruit with intermittent warming treatments show a decrease in electrolyte leakage (Liu *et al*., 2018; Balaguera-López *et al*., 2019).

In this experiment, clear differences were observed in firmness during storage (Table 2). The greatest reduction in firmness was observed with IW-4 d (13.98±1.74 N)

and IW-7 d (15.20±3.29 N) treatments. It has been suggested that this process is due to changes in carbohydrate metabolism and the action of hydrolases in the cell wall, such as polygalacturonase, which could be associated with an increase in ethylene production in fruit preserved in the cold and later removed to room temperature (Smith *et al*., 2002). Zhu *et al*. (2010) reported less firmness in peaches, explaining that the IW exposed fruit to one or more cycles of warm temperature during refrigerated storage, which also promoted ripening and quick softness.

The firmness in the Control, 1-MCP, and Ethylene treatments did not have significant differences and exhibited the highest value (Table 2). This indicated that, at low temperatures, these plant regulators did not have an effect, as reported by González *et al*. (2021) for 1-MCP in *Campomanesia lineatifolia* fruit stored at 2 °C. In avocado cv Hass fruit stored at 1 °C, Sivankalyani *et al*. (2015) also found no differences in firmness with 1-MCP.

The total soluble solids (TSS) were higher in the IW-4 d and IW-7 d treatments, which remained below 4%. The increased TSS in the warming treatments indicated a greater maturation process and sensory quality in the fruit (Rodrigues *et al*., 2020). In the Control, 1-MCP, and ethylene, the value was below 3%. This may be related to the reduced activity of 1-MCP and ethylene, as a result of the low storages temperatures (Blankenship and Dole, 2003). However, the treatments did not affect the total acidity (Table 2), in control, 1-MCP and ethylene, fruits showed low metabolism in the Krebs cycle by storage at 2 °C, while with the warming treatment the synthesis of organic acids may be temporarily increased, but also its degradation due to the greater respiratory metabolism in these fruits.

Table 2. Effect of Intermittent Warming for 4 days and 7 days, 1-MCP and Ethylene treatments on firmness, total solid soluble (TSS), and total acidity (TA) of "Hass" avocado fruits in storage at 2 °C for 29 days. Mean ± standard error (n=4).

	Storage		
Treatments	**Firmness (N)**	**TSS (%)**	**TA (%)**
Control	76.25±10 a	2.63±0.38 ab	0.16±0.04 a
IW-4 d	13.98±1.74 b	3.45±0.29 a	0.18±0.04 a
IW-7 d	15.20±3.29 b	3.60±0.30 a	0.16±0.2 a
1-MCP	72.53±22.2 a	1.88±0.14 b	0.15±0.04 a
Ethylene	85.24±3.01 a	2.03±0.26 b	0.14±0.02 a

Means followed by the same letter indicate no significant differences according to the Tukey test.

Shelf-life period

The fruit treated with intermittent warming 4 or 7 days, reached ripening at 4 days of the shelf-life, and for control fruit and those with ethylene was reached at 9 days. For 1-MCP, the ripening was reached at 14 days (Figure 3). The shorter shelf-life with intermittent warming may have been related to the increase in ethylene production in fruit preserved in cold and later removed to room temperature (Smith *et al*., 2002), a process that accelerates metabolism and maturation. Liu *et al*. (2018) indicated that IW might lead to some side effects in peaches and limit their shelf-life storage.

On the contrary, the avocados treated with 1-MCP lasted longer because this compound inhibits the perception and synthesis of ethylene (Balaguera-López *et al*., 2021) and, therefore, the ripening process. Daulagala *et al*. (2015) found that the 1-MCP treatment doubled the fruit shelf-life in avocado cv 'pollock', from 4 days to 9 days compared to the control.

A progressive increase in the respiration rate was observed. The highest respiration was obtained in the fruit subjected to ethylene, and the lowest was in the fruit with 1-MCP, although the respiratory rate in the intermittent warming treatments was not the highest in the shelf-life period. The fruit that had a higher value during storage had a shorter duration (Figure 3A). This representative increase in shelf-life was due to the

reconditioning of the fruit at room temperature in the storage period since this temperature change normalizes the metabolic processes, including respiration. Avocado ripening is associated with an increase in respiration rate, which is favored by the internal production or the external application of ethylene (Feng *et al*., 2000), which explains the higher respiratory rate in fruit subjected to ethylene. Ethylene has a close relationship with the respiratory process of the fruit. For this reason, 1-MCP applications are effective at reducing the respiratory rate of fruit since it affects biosynthesis, signaling, and processes that depend on ethylene during ripening (Blankenship and Dole, 2003; González *et al*., 2021), as was reported by some authors using avocados (Pesis *et al*., 2002; Munhuweyi *et al*., 2020).

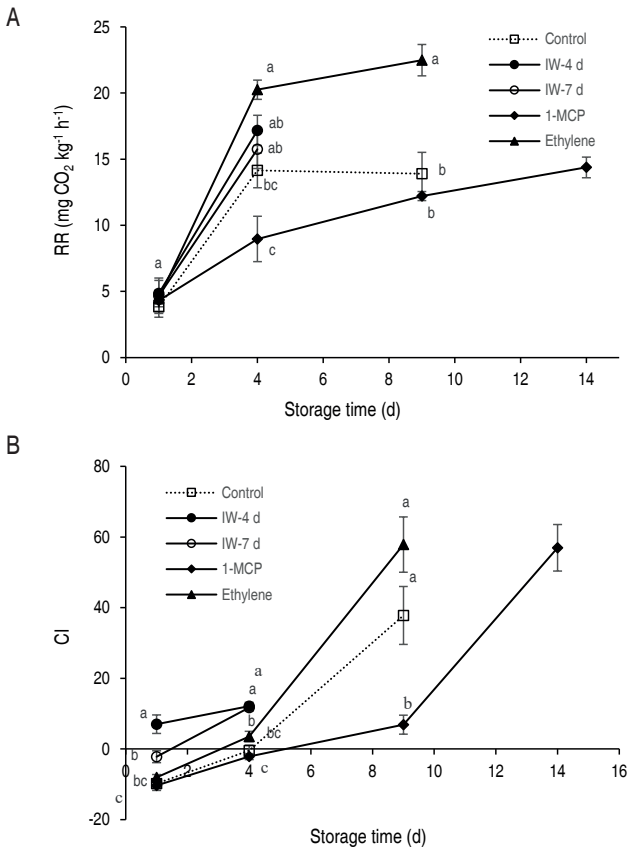

Figure 3. Effect of Intermittent Warming: IM-4 and IM-7, 1-MCP and Ethylene treatments on respiration rate (A) and color index (CI) of epidermis (B) of "Hass" avocado fruits in shelf-life (18 °C) after being stored 29 days at 2 °C. The vertical bars on each mean indicate the standard error (n=4). Means followed by the same letter indicate no significant differences according to Tukey's test (P<0.05).

The CI in the epidermis presented statistical differences throughout the shelf-life period. The highest index at the end of the shelf-life was obtained by fruit with ethylene (57.9), followed by fruit treated with 1-MCP. However, the CI of the fruit treated with intermittent warming had lower values (12.1) (Figure 3B). The pulp CI in the fruit with 1-MCP had the highest value (Figure 4A). These higher values in the color index were associated with browning, caused by chilling injury on the epidermis and in the pulp (Figure 5).

In the shelf-life period, 1-MCP retarded the avocado fruit ripeness, where firmness had values of 60.76±1.28 N (Table 3). This delay in softening was attributed to the inhibition of the enzymes involved in the degradation of the cell wall, such as cellulase and pectinmethyl esterase, polygalacturonase, 1,4-β-D-glucanase, β-galactosidase, exopolygalacturonase, endopolygalacturonase, and pectin esterase (Feng *et al*., 2000; Jeong *et al*., 2002). Thus, the firmness did not have significant differences between the Control, Ethylene, and IW treatments. Tomato

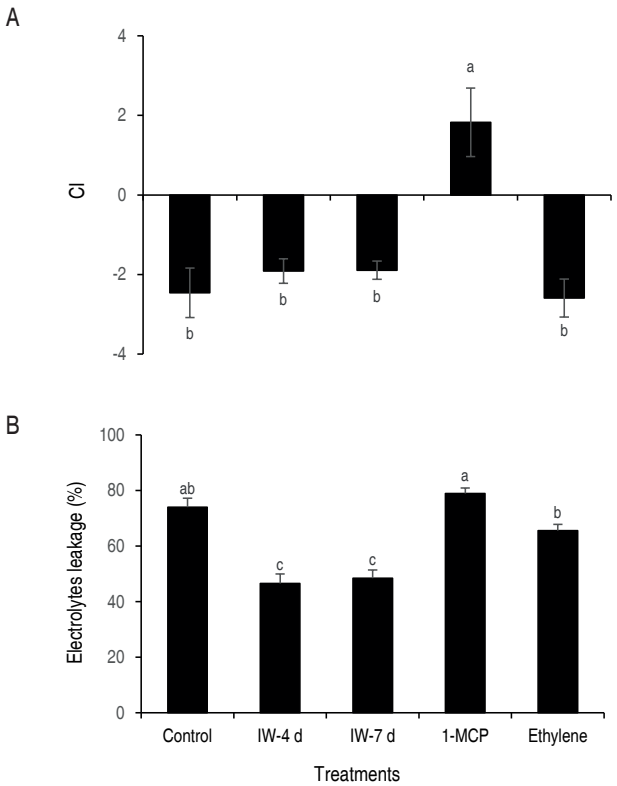

Figure 4. Effect of Intermittent Warming: IW-4 d and IW-7 d, 1-MCP and Ethylene treatments on the color index (CI) of pulp (A), and electrolytes leakage (B) of "Hass" avocado fruits in shelf-life (18 °C) after being stored 29 days at 2 °C. The vertical bars on each mean indicate the standard error (n=4). Means followed by the same letter indicate no significant differences according to Tukey's test (P<0.05).

fruit treated with 1-MCP was firmer than control, and the polygalacturonase activity was suppressed (Choi *et al*., 2008). In avocado cv Hass, 1-MCP delayed the loss of firmness and decreased the activity of polygalacturonase and cellulase enzymes; on the other hand, the application of ethylene had the opposite effect (Feng *et al*., 2000).

Table 3. Effect of Intermittent Warming IM-4 d and IW-7 d, 1-MCP and Ethylene treatments on firmness, total solid soluble (TSS), and total acidity (TA) of "Hass" avocado fruits in shelf-life (18 °C) after being stored 29 days at 2 °C. Mean ± standard error (n=4).

	Shelf-life		
Treatments	**Firmness (N)**	**TSS (%)**	**TA (%)**
Control	9.83±3,6 b[z]	3.38±0.14 ab	0.13±0.004 a
IW-4 d	6.23±1.0 b	3.38±0.31 ab	0.17±0.02 a
IW-7 d	11.37±0.25 b	3.60±0.21 a	0.15±0.01 a
1-MCP	60.76±1.28 a	2.63±0.19 b	0.13±0.01 a
Ethylene	10.13±2.09 b	3.45±0.15 ab	0.13±0.006 a

[z] Means denoted by the same letter do not significantly differ at P<0.05 according to the Tukey test.

TSS evaluation in the shelf-life, the fruit submitted to IW-7 d and 1-MCP had TSS values of 3.60±0.21% and 2.63±0.19%, respectively. The shelf-life period increased the SST content compared to the refrigeration period. This increase could be due to a high concentration of sugars, coming from the degradation of pectin, which is

more concentrated because of the fruit dehydration that results from an accelerated ripening process induced by a temperature change (Botía-Niño *et al*., 2008). Similar results were observed in curuba fruit (*Passiflora tripartita var. mollissima*) because of interrupted refrigerated storage (Botía-Niño *et al*., 2008). An increase in the concentration of soluble sugars depends on the production of ethylene, which explains the observations in the ethylene treatment and the IW treatments. 1-MCP can affect carbohydrate metabolism, which manifests as a slower increase in the concentration of soluble sugars. However, the TA was not significantly affected by the treatments (Table 3), indicating that the metabolism of organic acids at consumption maturity is not affected.

The electrolyte leakage had statistical differences (P<0.05). The intermittent heating treatments reduced this parameter, while the 1-MCP released more electrolytes (Figure 4B). Damage to the membrane during cold storage was due to increased lipid peroxidation, where unsaturated fatty acids are affected by reactive oxygen species (Ma *et al*., 2014; Liu *et al*., 2018). However, with the intermittent warming, the electrolyte leakage was lower in the shelf-life period (Figure 4B). In peaches, IW reduced electrolyte leakage and mitigated chilling injury by increasing antioxidant activity; 1-MCP also generated these same results (Liu *et al*., 2018).

Chillin injury index

High chilling injury index values in the Hass avocado fruit pulp were obtained in the 1-MCP treatment (P<0.05), without differences from the control, Ethylene and IW-7 d treatments. The fruits in the IW-4 d treatment obtained the lowest value (Figure 5A). In the epidermis, the highest index was obtained in the fruit with ethylene, 1-MCP and the control. The lowest index was observed in the fruit with IW-4 d and IW-7 d (Figure 5B). Liu *et al*. (2018) emphasized that, if the exposure to cold is finished and the temperature rises above the critical temperature, the tissue recovers from the metabolic imbalance, or the development of visible symptoms of damage is accelerated (if a long time has passed under the critical temperature). Therefore, these results showed that avocado cv. Hass is sensitive to chilling injury when stored at 2 °C (Figure 5). Sivankalyani *et al*. (2015) found chilling injury in avocado cv Hass fruit stored at 1 °C, which decreased with applications of 1-MCP. Woolf *et al*. (2020) reported apparent symptoms of critical chilling at 13 °C, including pulp browning.

The fruit treated with intermittent warming was efficient in reducing the chilling injury although not completely, which was more effective with IW-4d (Figure 5). Therefore, the thermal treatment may have generated tolerance to chilling in the avocado cv Hass fruit through the modulation of the antioxidant systems, which can prevent the accumulation of reactive oxygen spices, as was reported by Ma *et al*. (2014). Liu *et al*. (2018) indicated that IW was more effective in reducing chilling injury than 1-MCP in peaches stored at 2 °C. Likewise, Balaguera-López *et al*. (2019) reported that intermittent warming for 1 day at 18 °C and for 12 days at 2 °C reduced chilling injury in "Arrayana" mandarins.

In addition, treatments with intermittent warming for pear cv. "Nanguo" decreased peel browning after cold storage, while providing a higher ATP supply and energy charge, which were the result of the higher enzyme activity; this treatment also increased the gene expression of transcripts for ATP synthase, NADH dehydrogenase, and vacuolar proton-inorganic pyrophosphatase (Wang *et al*., 2018).

Studies on avocado cv. Strong have shown a partial response since fruit treated with 1-MCP showed a reduction in chilling injury in the pulp (Pesis *et al*., 2002), with no effect on browning of the epidermis (Woolf *et al*., 2003). However, in this study, there was a high estimation of chilling injury in the pulp and epidermis, which showed that the effect varied greatly according to the variety and dose of the product (Blankenship and Dole, 2003). However, the internal browning and development of reddish colorations in the pulp of apricots, peaches, or some varieties of plum, among others, increased significantly during cold storage when treated with 1-MCP (Liu *et al*., 2011; Wei *et al*., 2019). Although the theoretical basis by which sensitivity to chilling injury is increased is not known, a certain amount of ethylene would be necessary to alleviate the incidence of this damage associated with the immaturity of the product (Heyes, 2018; Wei *et al*., 2019).

The effects of ethylene and 1-MCP on chilling injury depend on the species and variety. Thus, ethylene treatments accelerate the symptoms of chilling injury in avocados (Pesis *et al*., 2002), and 1-MCP can reduce chilling injury in persimmon (Salvador *et al*., 2004).

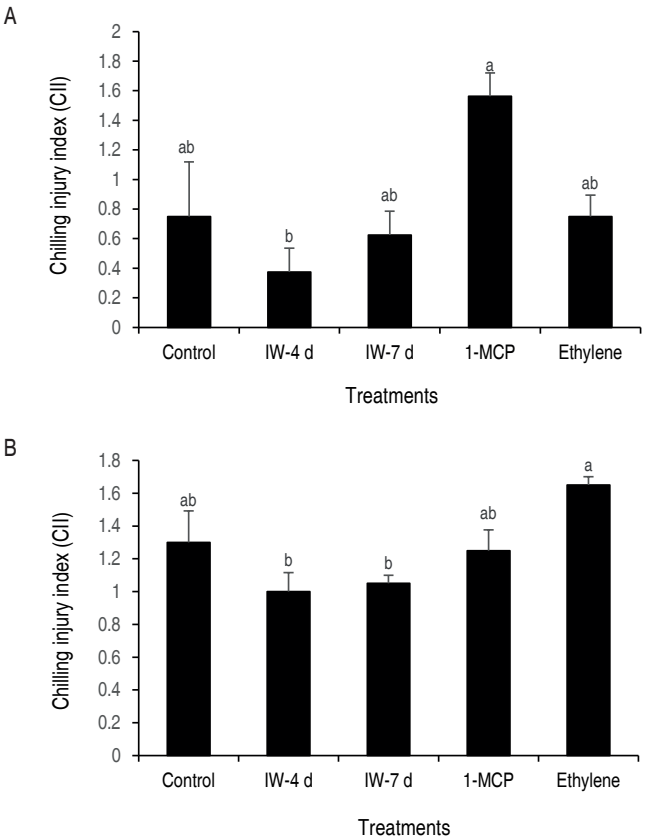

Figure 5. Effect of Intermittent Warming: IW-4 d and IW-7 d; 1-MCP and Ethylene treatments on chilling injury index of pulp (A), chilling injury of epidermis (B) of "Hass" avocado fruits in storage at 2 °C. The vertical bars on each mean indicate the standard error (n=4). Means followed by the same letter indicate no significant differences according to Tukey's test (P<0.05).

Therefore, although ethylene seems to be involved in the development of chilling injury in climacteric and non-climacteric fruit, the mechanisms and results of its actions do not seem to be the same in all species and varieties (Salvador *et al*., 2004).

CONCLUSIONS

Avocado fruits stored at 2 °C extend their shelf life, but some treatment should be used to alleviate chilling injury. The intermittent warming was favorable in the postharvest avocado cv. Hass because it reduced the chilling injury, mainly intermittent warming with a 4 days cycle; however, this treatment increased the weight loss, respiratory rate, epidermis color index, and electrolyte leakage during storage at 2 °C. In the shelf-life period, the intermittent warming resulted in a faster ripening process, even with the application of ethylene. 1- MCP is not recommended for use on avocado cv. Hass fruit when stored at 2 °C even though, it delays ripening, mainly during shelf-life, because it does not decrease chilling injury.

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Research article

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Effect of integrated plant nutrient management on indicators related to yield and productivity of spring barley (*Hordéum vulgáre*) under drought conditions in the growing season

Efecto del manejo integrado de nutrientes de las plantas en los indicadores relacionados con el rendimiento y la productividad de la cebada de primavera (*Hordéum vulgáre*) en condiciones de sequía en etapa de crecimiento

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Keywords:
Abiotic stress
Integrated plant nutrient management system
Localization of fertilizers
Mineral fertilizers
Stress protector

ABSTRACT

The agricultural production of the world is becoming increasingly vulnerable to extreme weather conditions, but adaptation to such conditions still suffers from a lack of integrated solutions and approaches that should cover relevant plant nutrition management issues, as well as technological mechanisms and tools. This study aimed to investigate the impact of fertilizer systems on yield indicators and the productivity of spring barley under arid growing conditions and determine the correlation between yield indicators and barley productivity to form a fertilization strategy for this crop under drought conditions. Two separate field experiments were conducted in 2018 in a small plot in six replications based on a long-term research field experiment on chernozem-type soil (black soil). As components of an integrated plant nutrient management system, the combined application of mineral fertilizers and stress protectors was used (for seed inoculation and foliar treatment), as well as the local application of mineral fertilizers at different depths (10-12 cm, 20-22 cm and at two depths simultaneously). The application of components of an integrated plant nutrient management system increased the chlorophyll content, leaf water content, and grain yield. These benefits of integrated fertilizer management led to significant improvement in grain yield. The maximum values of this indicator were noted for foliar treatment of plants by a stress protector at a rate of 1.0 L ha^{-1} (once per growing season) or 0.5 L ha^{-1} (twice per growing season) and for deep localization of mineral fertilizers (to a depth of 20-22 cm). The content of chlorophyll and bound water in the tissues of barley plants can be used as an indicator to determine the resistance of plants to arid growing conditions since these physiological characteristics are closely correlated with the yield of barley.

Palabras clave:
Estrés abiótico
Sistema integrado de gestión de la nutrición vegetal
Localización de fertilizantes
Fertilizantes minerales
Protector de estrés

RESUMEN

La producción agrícola del mundo se está volviendo cada vez más vulnerable a las condiciones climáticas extremas, pero la adaptación a tales condiciones todavía adolece de una falta de soluciones y enfoques integrados que deberían cubrir los problemas relevantes de gestión de la nutrición vegetal, así como los mecanismos y herramientas tecnológicos. El objetivo de este estudio fue investigar el impacto de los sistemas de fertilizantes en los indicadores de rendimiento y la productividad de la cebada de primavera en condiciones de crecimiento áridas y determinar la correlación entre los indicadores de rendimiento y la productividad de la cebada para formar una estrategia de fertilización de este cultivo en condiciones de sequía. En 2018 se llevaron a cabo dos experimentos de campo separados en parcelas pequeñas con seis repeticiones basadas en un experimento de campo de investigación a largo plazo en un suelo tipo chernozem (suelo negro). Como componentes del sistema de manejo integral de la nutrición vegetal, se utilizó la aplicación combinada de fertilizantes minerales y protectores de estrés (para inoculación de semillas y tratamiento foliar), así como la aplicación local de fertilizantes minerales a diferentes profundidades (10-12 cm, 20-22 cm y a dos profundidades simultáneamente). La aplicación de componentes del sistema integrado de gestión de la nutrición vegetal aumentó el contenido de clorofila, el contenido de agua de las hojas, producción de grano. Estos beneficios del manejo integrado de fertilizantes condujeron a una mejora considerable en el rendimiento de grano. Los valores máximos de este indicador se observaron para el tratamiento foliar de las plantas con un protector de estrés a una tasa de 1.0 L ha^{-1} (una vez por temporada de crecimiento) o 0.5 L ha^{-1} (dos veces por temporada de crecimiento) y para la localización profunda de fertilizantes minerales (hasta una profundidad de 20-22 cm). También se determinó que el contenido de clorofila y agua unida en los tejidos de las plantas de cebada se puede utilizar como indicadores para determinar la resistencia de las plantas en condiciones de crecimiento árido, dado que estas características fisiológicas están estrechamente relacionadas con el rendimiento de la cebada.

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Spring barley (*Hordéum vulgáre*) is an early ripening crop with a significant variety of forms. It withstands air drought better than other spring grains, providing sustainable yields. The grain of barley is widely used for food, feed, and brewing purposes. In terms of sown area, it ranks second among spring grain crops in Ukraine (Statistical Yearbook, 2020). The general demand of the state for barley grain significantly exceeds the level of modern production. A successful solution to this problem lies in the stable increase in its grain productivity. Barley has a short growing season; therefore, it reacts negatively to insufficient soil moisture during the period of active growth of leaf mass. Drought as abiotic stress mostly limits the growth and development of crops (Barnabás *et al*., 2008; Sehgal *et al.,* 2019). Water stress hinders growth by diminishing the water turgor of the plant cells, which adversely affects biochemical and physiological processes in plants (Liang *et al.,* 2019). One of the primary physiological consequences of water deficit is the prohibition of photosynthesis, because of a deficit of Ci (intercellular CO_2 concentration) as a result of chlorophyll destruction, stomatal closure, and disorder of the photochemical system (Liu *et al.,* 2016). Morgun *et al.* (2010) in their scientific review also noted, that suppression of plant growth under conditions of soil moisture deficit occurs mainly due to a decrease in their carbon dioxide balance (the difference between the absorption and loss of CO_2), which depends on the ratio of photosynthesis and respiration. Salehi (2016), Agami (2016), Manivannan *et al*. (2007), and Mamnabi *et al.* (2020) describe a deterioration of the physiological characteristics of plants, due to water deficiency, as a sign of oxidative stress damage. The production of reactive oxygen species (ROS) is a physiological response of plants to drought stress. The participation of ROS in the work of some signaling systems and the activation of genes involved in defense reactions has already been proven (Suzuki and Mittler, 2006; Kolupaev and Karpets, 2010).

Water deficit limits the physiological performance of plants by decreasing chlorophyll a (Chl a), chlorophyll b (Chl b), membrane stability index (MSI) (Ghassemi *et al.,* 2018), and chlorophyll content index (CCI) (Ghassemi-Golezani and Afkhami, 2018), the water content in plant tissues (Mamnabi *et al.,* 2020), and the supply of nutrients (N. P, K) (Rouphael *et al*., 2012).

For instance, one of the main reasons for the decrease in plant productivity under hydrothermal stresses conditions is the inhibition of photosynthesis, which quickly reacts to water deficit. It was found that the gas exchange of H_2O and CO_2 and cell growth are very sensitive to water deficit in the soil (Sadras, 1996). A slight decrease in soil moisture causes inhibition of the photosynthetic assimilation of carbon dioxide, primarily due to the partial stomatal closure. Chlorophyll concentration is considered to be a sensitive indicator of the state of the plant and the resistance of the genotype to water stress (Chernyad'ev, 1997). The chlorophyll content in plants is an important physiological parameter that characterizes the potential capacity of the photosynthetic apparatus. In general, the intensity of photosynthesis for plants under drought conditions plays an important role. It has already been established that changes in the temperature and water regimes of the leaf, which occur during atmospheric and soil drought, lead to a destruction of the fine structure of chloroplasts, the configuration of protein macromolecules, and the degree of aggregation of pigments adsorbed by the protein. The most important vital processes of plants are associated with photosynthesis, primarily mineral nutrition. It was found that during soil drought, with increasing water deficit, the chlorophyll content in all studied wheat varieties decreased by 13-15% (Kozhukhar *et al*., 2010). The use of different fertilizer systems can positively influence this indicator. It is shown in the examples of different crops that the application of fertilizers, biologicals, and other growth regulators has a positive effect on the chlorophyll content in the leaves, intensifying photosynthetic activity (Ahmadpour *et al*., 2016; Hosseinzadeh *et al*., 2018).

The state of water in plant tissues is also an important indicator of the plant's response to water deficiency. Fractional composition and ratio of water fractions (free, bound) also depends on species characteristics of plants, age, season, time of day, and conditions of mineral nutrition. Bound water supports the structure of colloids and ensures the functioning of enzymes, organelles, and cells. It is inactive and does not participate in the dissolution and transport of substances, but there is a direct relationship between the content of bound water and the resistance of plants to stress (Galasheva *et al*., 2013). Plant nutrition management can improve water conditions in plant tissues. Therefore, inoculation by

bio-fertilizer under water limitations increased the leaf water content (Kheirizadeh *et al*., 2016). This could be the result of enhancing root growth by indole-3-acetic acid (IAA) produced by bacteria (Marulanda *et al*., 2009). Also, vermicompost has a porous structure, more water holding capacity, organic ions, and plant hormones, so it can improve the leaf water content (Beykkhormizi *et al*., 2016). On the other hand, the improvement of water content in the leaf due to the use of fertilizers may be associated with an increase in the content of osmolytes such as soluble sugars.

In drought conditions, a lower transpiration rate reduces the transfer of nitrogen from plant roots to shoots, thereby limiting nutrient absorption (Rouphael *et al*., 2012). The availability and absorption of phosphorus and potassium also declines with decreasing soil moisture content (Marschner, 2012). However, the total annual precipitation has a minor effect on the change in available phosphates content in chernozem soil (black soil) compared to the nitrogen. In general, according to Sun *et al.* (2009), the synergism of moisture, nitrogen, and phosphorus for cereals, decreases in the following order: nitrogen and moisture> phosphorus and moisture> nitrogen and phosphorus. In such conditions, the use of optimal fertilizer systems can significantly increase the availability of important nutrients for plants.

In such conditions, the soil management approach must rely on an integrated plant nutrient management system (IPNMS), such as optimization of fertilizer application methods (in particular, localization methods), joint use of mineral fertilizers, and stress-protective preparations (plant growth regulators and physiologically active substances). Using these components of IPNMS can lead to better nutrients uptake and improve drought tolerance (Mittler, 2006). IPNMS is one of the basic pillars of sustainable agriculture. However, the relationship between fertilizer application optimization technologies and physiological yield indicators has not yet been established; it is not clear whether these indicators can be an important diagnostic value of plant responsiveness to drought growth conditions. Therefore, the aim of this study was to investigate the impact of fertilizer systems on yield indicators and productivity of spring barley under arid growing conditions and to determine the correlation between yield indicators and barley productivity of barley to form a strategy for fertilizing this crop under these conditions.

MATERIALS AND METHODS

Experimental conditions

Two field experiments were conducted in 2018 at the long-term research field experiment on chernozem (black soil) of the NSC "Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky" (State Enterprise "Experimental Farm "Grakivske", Kharkiv oblast, Ukraine, geographical coordinates: 49°46′N lat., 39°40′ E long). This experiment was located in a chernozem typical heavy loamy (Haplic Chernozems according to WRB classification) with the following characteristics: pH_{KI} 5.3; humus content 5.4% (it was determined by the Tyurin method); total nitrogen content 0.2% (it was determined by the Kjeldahl method), the content of mobile phosphorus and potassium 57 and 114 mg kg^{-1}, respectively (these were determined in acetic acid extract).

In the first experiment, the complex effect of stress-protective preparations (synthesized in the laboratory of Agrochemistry Department) and mineral fertilizers were studied (at a rate of 30 kg ha^{-1} for each element nitrogen, phosphorus, and potassium). Mineral fertilizers were used as an agrochemical background (control), inoculation of seeds was carried out by a stress protector for seeds (SPs) at a rate of 1.0 L t^{-1}, foliar treatment was performed by a stress protector for foliar (SPf) at a rate of 0.5 L ha^{-1} and 1.0 L ha^{-1}. The scheme of the field experiment is presented in full accordance with the options in Table 1. Foliar treatment was carried out once during the growing season in the tillering phase (BBCH 23) of barley or twice during the tillering (BBCH 23) and first node stage (BBCH 32). Stress protectors for seeds included a complex of trace elements, sodium tripolyphosphate, phytohormones, silicon, and liposam (a polysaccharide of the natural origin with sticky and moisture-retaining properties). Stress protector for foliar in addition to the above contained a complex of amino acids of the L-configuration and macronutrients in mineral form. The second experiment studied the effect of the depth localization of mineral fertilizers (at a rate of 60 kg ha^{-1} for each element nitrogen, phosphorus, and potassium). The fertilizers band was placed 4-5 cm away from the seed row at three different depths: 10-12 cm, 20-22 cm, and in two strips simultaneously (10-12 cm and 20-22 cm).

Table 1. Schemes of field experiments.

No. options	Field experiment No. 1	Field experiment No. 2
1	$N_{30}P_{30}K_{30}$ - (Control)	Control (without fertilizers)
2	Control + SPs (at rate 1.0 L t^{-1})	$N_{60}P_{60}K_{60\ (complex)}$ 10-12 cm
3	Control + SPs (at rate 1.0 L t^{-1}) + SPf (at rate 0.5 L ha^{-1} twice)	$N_{60}P_{60}K_{60\ (complex)}$ 20-22 cm
4	Control + SPf (at rate 1.0 L ha^{-1} once)	$N_{60}P_{60}K_{60\ (complex)}$ 10-12 cm and 20-22 cm
5	Control + SPf (at rate 0.5 L ha^{-1} twice)	$N_{60}P_{60}K_{60\ (fertilizer\ mixture)}$ 20-22 cm

The experiments were laid out as small plots in six replications; the area of each plot was 4 m^2. All technological operations (fertilization, sowing, loosening, weeding, harvesting, and crop accounting) were carried out manually except for tillage. In both experiments, seeds were sown at the end of March in about 2–3 cm in depth with a row spacing of 15 cm. Barley was harvested in the first week of July.

The weather conditions for the growing season of spring barley in 2018 were very unfavorable (Table 2). The air temperature in April-May and July 2018 was higher compared to the long-term average. At the same time, April-May and July 2018 were very dry. The listed features of weather conditions characterize the growing season of 2018 as arid at the beginning and at the stage of grain ripening.

Table 2. Weather conditions during the study period.

Month	Air temperature (°C)		Precipitation (mm)	
	2018	Average long-term	2018	Average long-term
April	12.4	11.0	12.9	34.5
May	19.9	17.9	15.9	38.9
June	21.6	21.9	43.5	42.2
July	23.0	21.9	28.7	50.2
mean	19.2	18.2	25.25	41.45

Measurements

Chlorophyll content. Measurement of chlorophyll content in the leaf was performed using a portable device SPAD-502 Plus (Konica Minolta, Japan). This method is a non-destructive measurement of plant chlorophyll. The SPAD-502 Plus enables quick and easy measurement of the chlorophyll content of plant leaves without damaging the leaf. The content of chlorophyll in barley leaves was determined once during the growing season at the beginning of booting (BBCH: 41).

Leaf water content. The main characteristics of the water regime of plants (free and bound water content) were determined using a portable refractometer RHB 0-90 (REF107, HT119) (Kelilong Electron, China) and by thermostat-weight method (total water content). Determination of water fractions or, as they are called, water forms, was carried out by the refractometric method, under the action of a hypertonic 30-40% sucrose solution according to the principle proposed by Dumansky (Chmeleva and Kucher, 2016). Determinations were carried out at 20 °C. Using this method, the free water content was found, and the bound water content was determined from the difference between total and free water. The content of the water regime of plants was determined once during the growing season at the beginning of booting (BBCH: 41).

N, P, K contents. The content of nitrogen, phosphorus, and potassium in barley grain was defined according to the method to determine the total forms of nitrogen, phosphorus, and potassium in one sample of plant material,

described in MVI 31-497058-019-2005. The essence of the method lies in the decomposition of organic matter in the sample in the presence of a catalyst with boiling sulfuric acid, which leads to the formation of ammonium salts. Separate determination of nitrogen, phosphorus, and potassium was carried out in the hydrolyzate using a spectrophotometer (Spekol). The N, P, and K contents in barley plants was determined after harvesting.

Grain yield. The barley plants were harvested in 4 m^2 of the middle part of each plot and the grains with about 14–17% moisture content were separated from siliques and weighed.

Statistical analysis

All the data were analyzed using STATISTICA 13.5.0.17. Differences among all experimental data were compared by employing the least significance difference (LSD) test at $P<0.05$ probability level.

RESULTS AND DISCUSSION

Chlorophyll content

Analysis of the data for two experiments showed a significant influence of components of IPNMS on chlorophyll of barley (Tables 3 and 4). In the first experiment, foliar treatment of plants by a stress protector at a rate of 1.0 L ha^{-1} (once per growing season) or 0.5 L ha^{-1} (twice per growing season) was the most effective. An increase in the chlorophyll content by 13.6-11.4 units was observed. The results of the second experiment (Table 3) showed a positive trend towards the accumulation of chlorophyll with an increase in the depth of localization fertilizers, however, within the least significant difference (LSD) at a significance level of 95%.

Table 3. Fractional composition of water and chlorophyll content in the tissues of spring barley plants using stress protectors.

Treatments	Total water content (%)	The content of individual fractions of water (%)		Chlorophyll content in plant tissues, (nmol cm^{-2})
		free water	bound water	
$N_{30}P_{30}K_{30}$ - agrochemical background (control)	64.4	55.4	9.0	35.3
Control + SPs (at rate 1.0 L t^{-1})	67.9	55.4	12.5	45.7
Control + SPs (at rate 1.0 L t^{-1}) + SPf (at rate 0.5 L ha^{-1} twice)	67.5	56.7	10.8	45.0
Control + SPf (at rate 1.0 L ha^{-1} once)	68.5	54.7	13.8	48.9
Control + SPf (at rate 0.5 L ha^{-1} twice)	68.1	55.1	13.0	47.0
LSD($P \leq 0.05$)	0.9	2.2	2.5	5.9

Table 4. Fractional composition of water and chlorophyll content in leaves of spring barley, depending on the depth of application and forms of fertilizers.

Treatments	Total water content (%)	The content of individual fractions of water (%)		Chlorophyll content in plant tissues, (nmol cm^{-2})
		free water	bound water	
Control (without fertilizers)	55.3	45.2	10.2	45.4
$N_{60}P_{60}K_{60\ (complex)}$ 10-12 cm	61.2	49.3	11.8	48.0
$N_{60}P_{60}K_{60\ (complex)}$ 20-22 cm	71.8	55.7	16.1	51.9
$N_{60}P_{60}K_{60\ (complex)}$ 10-12 cm and 20-22 cm	70.5	58.2	12.4	49.8
$N_{60}P_{60}K_{60\ (fertilizer\ mixture)}$ 20-22 cm	67.2	54.4	12.8	48.9
LSD ($P \leq 0.05$)	0.5	0.6	2.0	7.2

In treatments 1-4, mineral fertilizers were used in the form of compound NPK fertilizer, in treatment 5 - in the form of ammophos, ammonium nitrate and potassium chloride.

In addition, the combined analysis of the data from two experiments showed a close correlation (r=0.90) between the chlorophyll content in the leaves and the barley grain yield (Figure 1). These results indicate the possibility of using data on chlorophyll content in leaves as an indicator of the responsiveness of barley plants to arid growing conditions, as well as form a strategy for fertilizing barley for growing in drought conditions using the IPNMS components. To determine the tolerance of plants to water stress, chlorophyll content has already been introduced as an index (Hosseinzadeh *et al*., 2018).

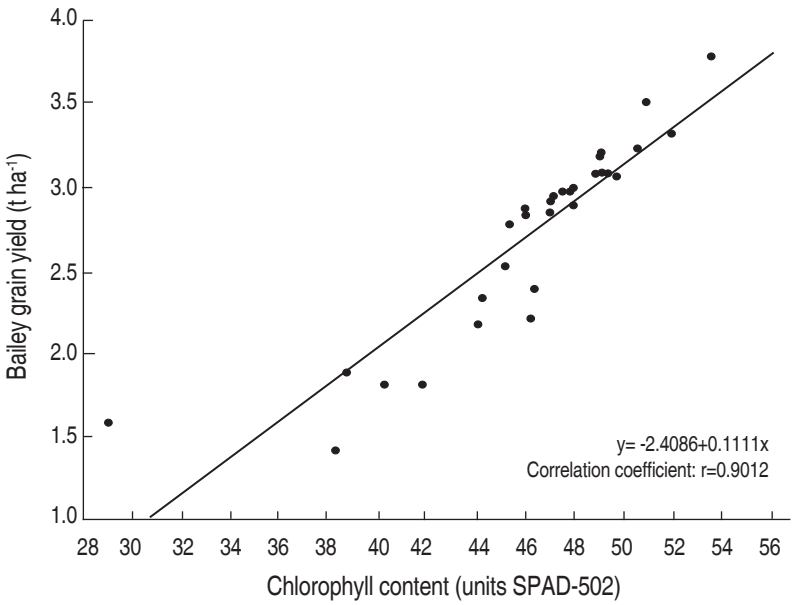

Figure 1. Relationship between indicators of chlorophyll content in plants and the barley grain yield.

In general, mineral nutrition conditions have an influence on the chlorophyll content in plants previously studied. Mamnabi *et al*. (2020) observed the highest chlorophyll content of rapeseed plants under drought stress when the fertilizer treatment was combined, which could be due to the positive effects of the fertilizer treatment on nitrogen and phosphorus supply. These macro-elements have the main role in manufacturing chlorophyll in leaves, cytosine, and oxine, and increase the physiological activity and total chlorophyll. The results of this study presented in TableS 3 and 4 confirm the observations made by Mamnabi *et al*. (2020) and validate the positive effect of additional fertilizers treatments and stress protector agents on the total amount of chlorophyll in barley plants.

Leaf water content

One of the conditions to determine the intensity and direction of physiological processes in plants and their tolerance to changes in the external environment is the degree of water supply and its state in plant tissues. In general, the use of the components of IPNMS had a positive effect on the content of bound water in the tissues of barley plants under drought conditions during the growing season (Tables 3 and 4). The maximum values of the content of bound water (4.8-5.9% higher compared to control) were noted during foliar treatment of plants by a stress protector at a rate of 1.0 L ha^{-1} and deep localization of mineral fertilizers (to a depth of 20-22 cm).

The combined analysis of the two experiments also allows to establish a close correlation between indicators of the content of bound water in plant tissues and barley grain yield (r=0.84) (Figure 2).

The state of water in plants depends on many conditions, one of which is mineral nutrition, optimization of which leads to the formation of the optimal balance of the fractional composition of water in plant tissues. Mamnabi *et al.* (2020) proved that the water content in the leaves of plants treated with biofertilizers was higher than the

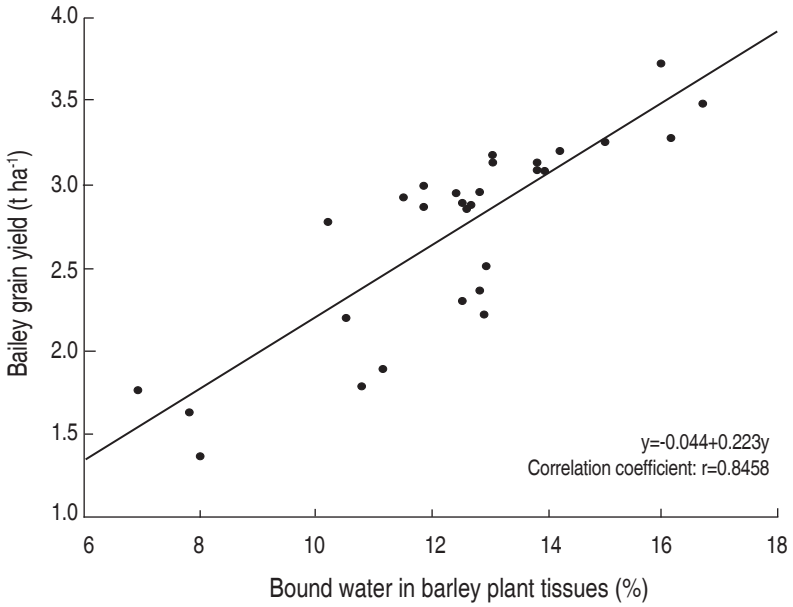

Figure 2. Relationship between indicators of bound water in plants tissues and the barley grain yield.

control, which is similar to the findings of this research (Tables 3 and 4).

N, P, K content

The use of stress protectors in combination with mineral fertilizers under drought conditions improved the content of nitrogen, phosphorus, and potassium in barley grain (Table 5). However, a component of IPNMS (such as the use of stress-protective preparations and mineral fertilizers in a complex) was significant only for the nitrogen content in plants; changes in the content of phosphorus and potassium were at the trend level.

Uptake and utilization of nutrients by barley plants were maximal with using foliar treatment of plants by a stress protector at a rate of 1.0 L ha^{-1} (Table 5). In comparison to the control, the uptake of nutrients was 38–48% higher, which is identical to the results obtained by Mittler (2006). He showed that the treatment of plants with stress protectors and growth stimulants increases the efficiency of using mineral nutrients since their uptake increases by 19-48%. Therefore, there was a positive influence of management of the soil nutrient regime on the efficiency of using nutrients by plants, and it coincides also with the opinion of Studer *et al*. (2017).

In general, the results of the current field experiment (Table 5) carried out in arid growing conditions of barley showed that the content of nutrients (P and K) in the plant cannot be used as an indicator of the application of IPNMS components performance.

Table 5. Content and uptake of nutrients (N, P, K) by the grain of spring barley.

Treatments	Content (%)			Uptake by barley (kg ha^{-1})		
	N	P	K	N	P	K
$N_{30}P_{30}K_{30}$ - agrochemical background (Control)	1.54	0.86	0.53	30.7	17.1	10.5
Control + SPs (at rate 1.0 L t^{-1})	1.81	0.91	0.56	42.6	21.4	13.2
Control + SPs (at rate 1.0 L t^{-1}) + SPf (at rate 0.5 L ha^{-1} twice)	1.81	0.91	0.52	41.9	21.2	12.1
Control + SPf (at rate 1.0 L ha^{-1} once)	1.72	0.90	0.54	48.8	25.4	15.4
LSD($P \leq 0.05$)	0.13	0.03	0.01	-	-	-

Grain yield

Compared to the control, all the components of IPNMS that were studied had a significant increase in grain yield (Tables 6 and 7). Foliar treatment of plants by a stress protector at a rate of 1.0 L ha^{-1} (once per growing season) or 0.5 L ha^{-1} (twice per growing season) turned out to be the most effective method for the combined use of mineral fertilizers and stress protectors (Table 6). The localization of the tape of mineral fertilizers at a depth of 20-22 cm maximized the yield of barley grain, which was almost twice as high as at a depth of 10-12 cm (Table 7). Combining NPK in one granule in compound mineral fertilizer turned out to be more effective (in treatments 1-4) than applying a mixture of simple and complex fertilizers (in treatment 5), when the granules of N, P, and K of fertilizers are separated from each other.

Table 6. Yield of spring barley grain by the application of stress protectors in combination with mineral fertilizers.

Treatments	Yield (t ha^{-1})	Increase (%)
$N_{30}P_{30}K_{30}$ - agrochemical background (Control)	2.00	-
Control + SPs (at rate 1.0 L t^{-1})	2.36	18.0
Control + SPs (at rate 1.0 L t^{-1}) + SPf (at rate 0.5 L ha^{-1} twice)	2.32	16.0
Control + SPf (at rate 1.0 L ha^{-1} once)	2.84	42.0
Control + SPf (at rate 0.5 L ha^{-1} twice)	2.69	34.5
LSD($P \leq 0.05$)	0.67	-

Table 7. Yield of spring barley grain by the depth of application and forms of fertilizers.

Treatments	Yield (t ha^{-1})	Increase (%)
Control (without fertilizers)	2.80	-
$N_{60}P_{60}K_{60\ (complex)}$ 10-12 cm	2.99	6.8
$N_{60}P_{60}K_{60\ (complex)}$ 20-22 cm	3.33	18.9
$N_{60}P_{60}K_{60\ (complex)}$ 10-12 cm and 20-22 cm	2.97	6.1
$N_{60}P_{60}K_{60\ (fertilizer\ mixture)}$ 20-22 cm	2.87	2.5
LSD($P \leq 0.05$)	0.25	-

In treatments 1-4 mineral fertilizers were used in form of compound NPK fertilizer, in treatment 5 – in form of ammophos, ammonium nitrate and potassium chloride.

Improving grain yield of barley plants by using components of IPNMS was related to higher chlorophyll and concentrations of N, bound water content in plants tissues (Table 3 and 4). In general, the amount of chlorophyll increased by enhancing the amount of nitrogen available to the plant and followed by the ability to absorb sunlight and produce more assimilates, and finally increasing the growth and yield in barley grain by 16-42% (Table 3 and 4), which was also confirmed by Salehi *et al.* (2016) and Sorokin *et al.* (2017). They reported that the application of various stress protectors of adaptive action increased the yield of grain crops by 200-400 kg ha^{-1}, in particular, winter wheat by 15-22% and spring barley - by 50-73%.

CONCLUSIONS

The use of components of IPNMS, such as the combined application of mineral fertilizers and stress-protective agents, and deeper localization of mineral fertilizers, significantly impact the yield indicators and the productivity of spring barley under arid growing conditions. In particular, it resulted in higher chlorophyll content by 13.6-11.4 units, bound water in plants tissues by 4.8-5.9%, uptake of nutrients by plants by 38–48%, and barley grain yield by 16-42%.

A strong positive correlation was found between the content of chlorophyll (r=0.90), bound water (r=0.84) in plants, and

the yield of spring barley, which confirms the important diagnostic value of these physiological characteristics for optimizing fertilizer application technologies, especially during the droughty growing season.

The strategy for fertilizing spring barley for growing in drought conditions includes the following components of an integrated plant nutrition management system: using a stress protector at a dose of 1.0 L ha^{-1} (once during the growing season) or 0.5 L ha^{-1} (twice during the growing season) in combination with mineral fertilizers; localization of the band of mineral fertilizers at a depth of 20-22 cm.

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Influence of fertilization on the crop rotation productivity and the balance of essential nutrients in the soil

Influencia de la fertilización en la rotación de cultivos de campo sobre su productividad y el equilibrio de nutrientes esenciales en el suelo

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ABSTRACT

The article shows the influence of different rates and combinations of mineral fertilizers on the balance of essential nutrients in the four-field crop rotation (winter wheat, corn, spring barley, and soybean) during eight years in the context of incorporation into the soil or removal of a non-commercial part of the yield of the field. The stationary field experiment was performed on the black podzolized heavy loamy soil of the Right-Bank Forest-Steppe. The experiment scheme included 11 variants of combinations and separate applications of mineral fertilizers as well as control variants without fertilization. It has been established that in the annual removal of nutrients together with grain harvest nitrogen makes up the biggest share (64.4–149.9 kg ha^{-1}), then phosphorus – 21.1–51.4 kg ha^{-1} depending on the fertilizing in the crop rotation. The variant of the experiment $N_{110}P_{60}K_{40}$ provides the optimal intensity of the balance of nitrogen, phosphorus, and potassium (103, 122, and 111%, respectively) when a non-marketable part of the yield is left for fertilizing in the field. When this part of the yield is removed from the field there is a deficit balance of nitrogen, phosphorus, and potassium with the intensity (76, 76, 61%, respectively) even in the variant with the annual average application of $N_{110}P_{60}K_{80}$. The use of non-marketable agricultural products in crop rotation for fertilization and the average annual application of $N_{110}P_{30–60}K_{40–80}$ allows compensation for the losses of nitrogen 24%, phosphorus 33%, and potassium 71%.

RESUMEN

Palabras clave:
Chernozem lúvico
Nitrógeno
Fósforo
Potasio

El artículo muestra una influencia de los primeros ocho años de un experimento a largo plazo de diferentes dosis y combinaciones de fertilizantes minerales sobre el equilibrio de nutrientes esenciales en la rotación en cuatro años de cuatro campos (trigo de invierno, maíz, cebada de primavera, soja) en el contexto de la incorporación en el suelo o la eliminación de la parte no comercial del rendimiento del campo. El experimento de campo se implantó en el suelo negro podzolizado pesado franco de las Bosques-Estepas de la orilla derecha. El esquema experimental incluye 11 variantes de combinaciones y aplicación separada de fertilizantes minerales, así como la variante de control sin fertilización. Se ha establecido que en la eliminación anual de nutrientes junto con la cosecha de granos, el nitrógeno constituye la mayor parte (64,4–149,9 kg ha^{-1}), luego el fósforo – 21,1–51,4 kg ha^{-1} dependiendo de la fertilización en la rotación de cultivos. La variante del experimento $N_{110}P_{60}K_{40}$ proporciona la intensidad óptima del equilibrio de nitrógeno, fósforo y potasio (103, 122 y 111%, respectivamente) cuando una parte no comercializable del rendimiento se deja para fertilización en el campo. Cuando esta parte del rendimiento se elimina del campo, hay un déficit de nitrógeno, fósforo y potasio con la intensidad (76, 76 y 61%, respectivamente) incluso en la variante con la aplicación media anual de $N_{110}P_{60}K_{80}$. El uso de productos vegetales no comercializables de la rotación de cultivos para la fertilización y la aplicación media anual de $N_{110}P_{30–60}K_{40–80}$ permite compensar las pérdidas de nitrógeno en un 24%, fósforo en un 33% y potasio en un 71%.

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Nowadays the application of fertilizers is one of the most significant parts of agricultural technologies (Šimanský and Jonczak, 2019; Novak *et al*., 2019). A long-term application of fertilizers increases the radioactivity of the soil due to the content of ^{40}K and ^{226}Ra, however, this radiation is safe for human health (Hospodarenko *et al*., 2019). Besides, the application of fertilizers improves the quality of the grain, which is important for the production of high-quality products (Hospodarenko and Liubych, 2021; Osokina *et al*., 2020; Petrenko *et al*., 2017).

The problem of soil fertility in Ukraine remains acute because of the large areas of plowed lands, drastic increase of landowners and land users, whom do not follow science-based crop rotation, and soil tillage, which leads to degradation processes: over-compactness, erosion (Medviediev *et al*., 2018). In addition to this, the economic crisis caused a considerable decrease in the level of using mineral fertilizers, which worsen the problem of soil fertility preservation.

Therefore, this involves the issue of developing such fertilizing systems for different soil and climate conditions that provide high productivity of plants with the simultaneous restoration of soil fertility. That is the reason why it is necessary to develop energy-saving and cost-effective fertilizing systems with high ecological effects, whose application would be expedient not only for large successful but also for small-size farm enterprises.

The efficiency of using organic and mineral fertilizers under field crops on the black soils was researched in the previous studies (Hamayunova and Filipiev, 1997; Ivanina, 2012; Lopushniak, 2015; Poliovyi, 2007). However, these experiments were carried out, as a rule, in different soil and climate conditions, under different structures of 8-12-field crop rotations and with the removal of straw from the field to meet the needs of animal breeding, and under a low level of yielding capacity of crops.

At present, most farms are involved in plant production, and as a result, manure is absent in the circulation of nutritional substances. Their specialization, change in farmland structure and nature protection aspects influence the intensity and approaches of crop fertilization system. The application of agricultural technologies that provide renewal of soil fertility and prevent pollution are of primary importance among the measures aimed at achieving a neutral level of soil degradation in Ukraine (Baliuk *et al*., 2018).

It is especially important to establish regional parameters of nutrient balance considering the type of crop rotations and the level of their productivity as well as optimal saturation of arable land with organic and mineral fertilizers. The efficiency of using fertilizers depends on their application rates in the first place. When calculating the optimal rate of fertilizers attention should be paid to the biological properties of crops and the planned level of yielding capacity, climatic conditions and soil fertility, the level of agrotechnology, the succession of crops in the rotation crop, and their saturation with fertilizers, forms of fertilizers, the method and timing of their application, and other factors. Therefore, determining the doses of fertilizers is one of the complicated issues of the current agronomical science and practice (Marschner, 2012; Hospodarenko *et al*., 2019). In addition to this, it has been established that it is possible to achieve a high yielding capacity of crops under regular application of fertilizers, and by adding considerably lower rates of fertilizers, which can be explained by the after-action of fertilizers applied under previous crops in the crop rotation (Šimanský, 2016).

Calculating the nutrient balance makes it possible to establish the direction of the processes in the system soil-fertilizer-plant, to determine the parts, surplus, or the deficit of certain nutritional elements of plants. Conducting such research in dynamics makes it possible to develop recommendations on the planned regulation of nutrient balance and humus in the soil (Bohdevych, 2006). Under a positive balance of nutrients, it is important to consider the necessity to increase soil fertility as well as the threat of pollution of the soils and water sources (Asif *et al*., 2019).

References provide the data about the expenses of nutrients on the formation of marketable products and the corresponding amount of non-marketable products, according to which is possible to determine the general need of a crop in nutrient elements for the formation of planned yields.

However, the relative removal of nutrients even in one crop changes considerably depending on the soil and climate conditions, the size of the yield, the correlation

between marketable and non-marketable produce in the yield, the quality of yield, specific features of varieties and hybrids (Ivanina, 2012).

It is considered that to make balance calculations when non-marketable produce is not removed from the field, it is worth using the index of potassium removal only by the main produce. To calculate the rates of fertilizers for planned yields it is reasonable to use the data about the expenses of potassium on the formation of the unit of the main product with the account of the necessary amount of non-marketable produce (Klochko and Syryi, 2011). It can be explained by the fact that the requirement potassium of plants can be provided up to 50% from the supplies below plowed soil layers (Hrckova *et al.*, 2018).

Data dealing with the productivity of short-term field crop rotations on the black soils which, depending on the level of fertilizing, are not sufficient. The fraction of every crop in short-term rotations increases, which influences the use of nutrients in the soil and fertilizers. The common rule that the higher the rates of fertilizers are, the higher the yields of crops, is not relevant in the conditions of the energy crisis. Closer determination of quantitative features of the balance in the soil-fertilizer-plant system is relevant in different soil and climate conditions with the account of the rates of fertilizers and combination of nutrients with the intention of developing practical recommendations on the optimization of nutritional regime of the soil and increase the productivity and protect the environment.

Therefore, this research aimed to evaluate the balance of the main nutrients in the black podzolized heavy loamy soil in the Right Bank Forest-Stepp of Ukraine under different rates and combinations of mineral fertilizers in a short-term crop rotation when a non-marketable part of the yield is incorporated into the soil or removed from the field and to validate the optimal saturation with fertilizers to restore soil fertility and obtain stable yields.

MATERIALS AND METHODS

The stationary field experiment was carried out at the Uman National University of Horticulture (certificate of the National Academy of Agricultural Sciences No. 87) (Stationary field experiments of Ukraine, 2014) in the Right-Bank Forest Steppe of Ukraine with Greenwich geographical coordinates 48°46' of northern latitude and 30°14' of eastern longitude. The experiment was started in 2011. The following crops were cultivated in the four-field crop rotation: winter wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), spring barley (*Hordeum vulgare* L.), and soybean (*Glycine max* Moench.). The field experiment aims to establish the efficiency of the action of different types, rates, and proportions of mineral fertilizers on the yielding capacity and quality of grain and seeds of field crops, and fertility of the black soil. The scheme of the experiment includes 11 variants of combinations and separate applications of mineral fertilizers including the control variant without fertilizers (Table 1).

Table 1. Design of application of fertilizers in the experiment.

Variant of the experiment: average rates of nutrients in the crop rotation (kg active substance ha^{-1} per year)	Application of fertilizers in the crop rotation			
	Winter wheat	Corn	Spring barley	Soybean
N_{55}	N_{75}	N_{80}	N_{35}	N_{30}
N_{110}	N_{150}	N_{160}	N_{70}	N_{60}
$P_{60}K_{80}$	$P_{60}K_{80}$	$P_{60}K_{110}$	$P_{60}K_{70}$	$P_{60}K_{60}$
$N_{110}K_{80}$	$N_{150}K_{80}$	$N_{160}K_{110}$	$N_{70}K_{70}$	$N_{60}K_{60}$
$N_{110}P_{60}$	$N_{150}P_{60}$	$N_{160}P_{60}$	$N_{70}P_{60}$	$N_{60}P_{60}$
$N_{55}P_{30}K_{40}$	$N_{75}P_{30}K_{40}$	$N_{80}P_{30}K_{55}$	$N_{35}P_{30}K_{35}$	$N_{30}P_{30}K_{30}$
$N_{110}P_{60}K_{80}$	$N_{150}P_{60}K_{80}$	$N1_{60}P_{60}K_{110}$	$N_{70}P_{60}K_{70}$	$N_{60}P_{60}K_{60}$
$N_{110}P_{30}K_{40}$	$N_{150}P_{30}K_{40}$	$N_{160}P_{30}K_{55}$	$N_{70}P_{30}K_{35}$	$N_{60}P_{30}K_{30}$
$N_{110}P_{60}K_{40}$	$N_{150}P_{60}K_{40}$	$N_{160}P_{60}K_{55}$	$N_{70}P_{60}K_{35}$	$N_{60}P_{60}K_{30}$
$N_{110}P_{30}K80$	$N_{150}P_{30}K_{80}$	$N_{160}P_{30}K_{110}$	$N_{70}P_{30}K_{70}$	$N_{60}P_{30}K_{60}$

In the variant of the experiment with an average rate of nutrients in the crop rotation per hectare $N_{110}P_{60}K_{80}$, the total (100%) compensation with fertilizers of average annual removal of the nutrients by the crops in the crop rotation is planned. The scheme of the experiment was developed in such a way that it could be possible to determine the opportunity to decrease the rates of certain types of mineral fertilizers. The placement of the variants in the experiment was successive. Performance of the experiment simultaneously on four fields provided annual data about the yielding capacity of all crops in the four-field crop rotation. Fertilizers application was replicated three times in the crop rotation deployed in space and time. The total area of the experimental plot was 110 m^2, and the accounting area was 72 m^2. Phosphorus (granulated superphosphate) and potassium (potassium chloride) fertilizers were applied during fall tillage, nitrogenous fertilizers (ammonium nitrate) during pre-sowing cultivation, and fertilizing of winter wheat.

The soil on the experimental plot is the luvic chernozem (World Reference Base for Soil Resources, 2014) heavy loamy soil on loess with 3.8% of humus content, pH_{KCl} – 5.7, the content of nitrogenous hydrolyzed compounds by the Cornfield method was 105 mg kg^{-1}, the content of mobile compounds of phosphorus and potassium by Chirikov method, was increased 106 mg kg^{-1} and 132 mg kg^{-1}, respectively.

Characteristics of genetic horizons of the luvic chernozem

He (0–42) – humus, slightly illuvial, dark-grey horizon with homogeneous humus content, dispersed fine-clodded structure, consolidated subsurface permeated with small roots and noticeable transition. Humus content was 3.8%, pH_{KCl} – 5.7.

Hpi (42–70) –slightly illuvial, dark-brown with the shade of parent material, small nut-like structure permeated with roots, with inhomogeneous humus content and noticeable transition. Humus content was 3.1%, pH_{KCl} – 5.9.

PIh (70–100) – slightly moistened illuvial horizon, dark yellow, nut-columnar structure with a transition to columnar, insignificant heterogenous. Humus content was 2.0%, pH_{KCl} – 7.0.

Pi(h) (100–120) – slightly illuvial, dark-yellow in color, with a prism-like structure, inhomogeneous insignificant humus content, and gradual transition to the parent rock, pH_{KCl} – 7.0.

Pk (120) – loess rock, brownish-yellow with fine texture, carbonates in the form of mold, veins, and individual clusters. The location of carbonates is at the depth of 120 cm, pH_{KCl} – 7.1.

Surface tillage was used (stubble disking after soybean harvesting was conducted twice) in the agrotechnology of winter wheat. Autumn tillage was applied on the corn, barley, and soybeans agrotechnology crops, including stubble peeling after stubble harvest and plowing at the depth of 28–30 cm

.

The amount of rainfall was by 1–4% higher over three years, it was 4–11% lower over the rest of the years compared to the average long-term index (Table 2). The air temperature was higher (2–7%) than the long-term average except for 2011.

The grain was harvested by combined harvesters. The accounting of the harvest of non-marketable produce was conducted by the method of the trial sheaf. Non-marketable part of the harvest of the crop rotation plants (straw, stems) was left in the field for fertilizing.

Table 2. Weather conditions throughout research (according to the Uman meteorological station)

Index	Year of the research								Long-term average index (1991–2020)
	2011	2012	2013	2014	2015	2016	2017	2018	
Rainfall, mm	593	584	555	608	527	509	524	601	586
Temperature (°C)	8.6	9.1	9.4	9.0	9.3	9.9	9.0	9.2	8.8

To simplify the calculations of nutrient balance the number of balance items was reduced both in terms of their supply and removal. Thus, the amount of nitrogen that comes into the soil from the atmosphere with precipitation and seeds is fixed by freely existing microorganisms was equal to its total expenses as the result of washing out, erosion and weathering. The total amount of phosphorus and potassium that come from the atmosphere and seeds was equal to the losses from erosion and washing out.

The content of nitrogen, phosphorus, and potassium in plant samples was determined after acid digestion (Yeshchenko *et al.*, 2014). To evaluate the strength of connection among the investigated factors the coefficient of correlation was used according to the scale of R. E. Chaddock (Chaddock, 1952). Statistical data processing was carried out by using the software Microsoft Excel 2010 and STATISTICA 10.

RESULTS AND DISCUSSION

Crop rotation can work effectively only under conditions where substances and energy are returned to circulation, which creates optimal nutrition conditions for plants to obtain high yields. The research has shown that the regular application of different rates and combinations of mineral fertilizers in the field crop rotation improves considerably its productivity (Figure 1). The application of nitrogen-potassium, nitrogen-phosphorus, and complete mineral fertilizers influenced the productivity of the field crop. On average, the application of nitrogenous fertilizers increased the productivity of crops rotation by 1.5 times, phosphorus-potassium by 1.3 times, nitrogen-potassium by 1.7, nitrogen-phosphorus by 1.8 times, and complete mineral fertilizer by 1.9 times.

Long-term application of N_{55} increased the productivity of the crop rotation by 1.4 times compared to the variant

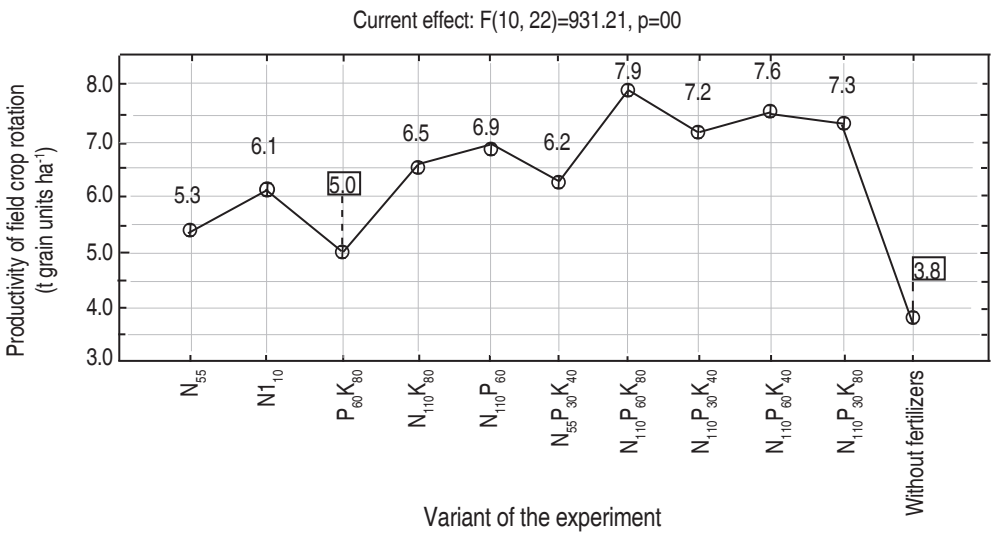

Figure 1. Productivity of field crop rotation under different fertilizers in the period from 2011 to 2018.

without fertilizers (Figure 2). Application of $N_{55}P_{30}K_{40}$ increased this index by 1.6 times compared to the plots without fertilizers and by 17% compared to the variant with N_{55}. Application of the doubled rate of nitrogen fertilizers increased the productivity of the crop rotation by 1.6 times compared to the variant without fertilizers. The productivity of the crop rotation, in this case, was 14% higher compared to the variant N_{55}. The application of complete mineral fertilizer ($N_{110}P_{60}K_{80}$) increased the productivity of the crop rotation by 2.1 times compared to the control and by 29% compared to the application of nitrogen fertilizers (N_{110}). Application of the complete mineral fertilizer (variants with the application N_{110} at the background of different proportions of phosphorus-potassium fertilizers) provided the highest productivity of the crop rotation (7.18–7.85 t ha^{-1} of grain units). Application of nitrogen-potassium and nitrogen-phosphorus fertilizers increased this index only by 6–14% compared to the variant N_{110}.

The productivity of the crop rotation under the application of phosphorus-potassium fertilizers was considerably lower compared to the other variants of fertilizing.

At the international conference in Uganda, the agreement was reached that the negative balance of nutrients is one of the main indexes of soil degradation (Bekunda and Manzi, 2003). The value of the maximum-permissible deficit of nutrition element depends on the content of its mobile compounds in soil and the level of productivity of a certain crop rotation. It is considered that for most types of soil there is no necessity for complete compensation of removed potassium on average during the rotation cycle (Prokoshev and Deriugin, 2000; Tobiašová *et al.*, 2013).

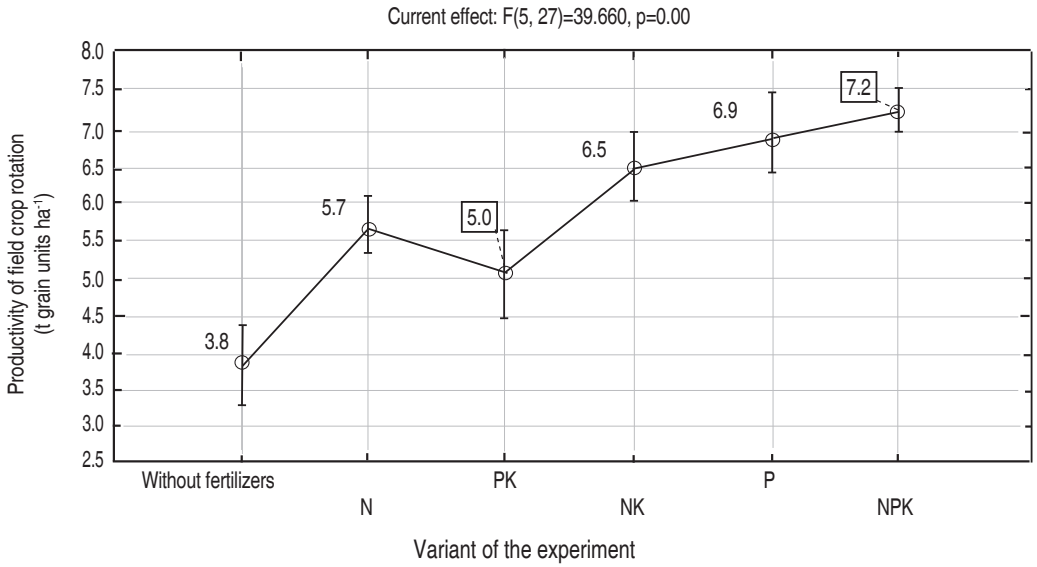

Figure 2. Productivity of field crop rotation under different fertilizers, their rates and proportions in the period from 2011 to 2018

Calculation of the nitrogen balance is used as the tool to evaluate the efficiency of fertilizers application and the corresponding ecological burden on the environment (Tarariko, 2011). The efficiency of its use in plant production is calculated as a share of nitrogen in the gathered harvest from its entering into the soil together with mineral and organic fertilizers, precipitation and the fixation of atmospheric nitrogen, etc. If this share exceeds 100%, the quality of the soil decreases.

Table 3 shows the balance of essential nutrients in the soil of the field crop rotation and its intensity. Taking into account the symbiotic atmospheric nitrogen fixed by soybean, the application of 110 kg of active substances of nitrogen fertilizers per hectare of crops rotation area provided 100–126% of the intensity of nitrogen balance on average during two rotation cycles depending on the experimental variant. It is necessary to mention that with the improvement of phosphorus and potassium nutrition of plants, the index of nitrogen balance intensity decreases. When the share of soybean is 25% in the structure of the crop rotation, the removal of nitrogen together with the main products by all crops was covered almost threefold (Hospodarenko *et al.*, 2019).

For the black soils in Moldova, the scheme was suggested that compensates for the removal of nitrogen by 26% due to organic fertilizers – manure and composts; 14% due to the fixation of atmospheric nitrogen by lentils, 48% due to the application together with mineral fertilizers. In this case, a 12% of nitrogen deficit is acceptable (Ivanina, 2012). The drawback of this approach is the coverage of nitrogen deficit due to manure by 26% which is explained by the decline in animal breeding.

The calculations showed that in the variant $N_{110}P_{60}K_{80}$ with 100% intensity of nitrogen balance during two rotation cycles in the crop rotation, its removal was

compensated by the application of nitrogen fertilizers (73%) and by symbiotic nitrogen fixation by soybean (27%).

Thus, optimal nitrogen balance with 100–107% intensity was achieved when nitrogen was applied annually with mineral fertilizers 110 kg ha^{-1} in different combinations with phosphorus and potassium ($P_{30–60}K_{40–80}$).

Phosphorus balance in arable farming is of special practical value for the black soils (Nosko, 2017). Its circulation is simpler than that of nitrogen because its components are only soil and plants. According to Kramariov and Kramariov (2018), to increase the fertility of the soil it is necessary to reach 180–200% of the intensity of phosphorus balance, which is provided by the annual application of 60–80 kg ha^{-1}.

Table 3. Balance of essential nutrients in the soil of the field crop rotation and its intensity provided that a non-marketable part of the yield is left in the field in the period from 2011 to 2018.

Balance item	Variant of the experiment										
	Without fertilizers	N_{55}	N_{110}	$P_{60}K_{80}$	$N_{110}K_{80}$	$N_{110}P_{60}$	$N_{55}P_{30}K_{40}$	$N_{110}P_{60}K_{80}$	$N_{110}P_{30}K_{40}$	$N_{110}P_{60}K_{40}$	$N_{110}P_{30}K_{80}$
Nitrogen											
Supply (kg ha^{-1})	21	81	139	26	142	144	85	150	144	149	145
Removal (kg ha^{-1})	64	95	110	87	119	129	111	150	135	144	138
Balance (kg ha^{-1})	-44	-14	29	-61	24	15	-26	0	9	4	7
Balance intensity (%)	32	85	126	30	120	112	77	100	107	103	105
Phosphorus											
Supply (kg ha^{-1})	–	–	–	60	–	60	30	60	30	60	30
Removal (kg ha^{-1})	21	30	34	31	36	45	38	51	44	49	45
Balance (kg ha^{-1})	-21	-30	-34	29	-36	15	-8	9	-14	11	-15
Balance intensity (%)	–	–	–	194	–	133	79	117	69	122	67
Potassium											
Supply (kg ha^{-1})	–	–	–	80	80	–	40	80	40	40	80
Removal (kg ha^{-1})	17	23	26	24	31	32	29	38	33	36	35
Balance (kg ha^{-1})	-17	-23	-26	56	49	-32	11	42	7	4	46
Balance intensity (%)	–	–	–	336	261	–	137	212	121	111	232

However, there is an opinion that with the improvement of the phosphorus regime in black soils the rate of phosphorus fertilizers can be decreased because excessive phosphorus nutrition decreases the availability of some microelements to plants and the quality of agricultural produce.

In Belarus, the system of applying mineral fertilizers implies the returning of nutrients, compensating for their removal along with the harvest and provides a gradual increase of the content of mobile compounds of phosphorus and potassium to 200–300 mg kg^{-1} of soil. In this case, the application of phosphorus and potassium fertilizers should compensate by 100% the removal of these elements by the planned yielding capacity of crops on the soils with the content of mobile compounds of phosphorus and potassium lower than optimal value – 120, and on the soils with their content higher than optimal value by 50% removed together with the yields (Lapa and Tsybulko, 2018). Calculations have shown that alongside the improvement of mineral nutrition of plants due to the application of fertilizers the removal of phosphorus from the soil together with the yield increases more than two-fold (Table 3). The application of phosphorus fertilizers at the rate of 30 kg ha^{-1} at the background of $N_{55}K_{40}$ on average per ha^{-1}

of crop rotation does not provide a positive balance of phosphorus. The intensity of phosphorus balance at the level of 117–133% was formed in the variants $N_{110}P_{60}$, $N_{110}P_{60}K_{40,}$ and $N_{110}P_{60}K_{80}$. In the background of applications $N_{110}K_{40}$ and $N_{110}K_{80}$, according to this study, the balance of phosphorus is provided by the application of 49 and 51 kg ha^{-1} respectively.

Luvic chernozem has the most favorable potassium regime. In 70% of areas, the soils in Ukraine provide an increased and high level of nutrition for plants with potassium. To maintain a potassium regime, it is necessary to apply 35 kg ha^{-1} of arable soil annually together with fertilizers (Medviediev *et al*., 2018). On the black soils of the Forest Steppe, the calculated rate of potassium fertilizers in crop rotations with the organic and mineral fertilizing systems could be reduced by 20%. In the areas with irregular rainfall, the return of removed potassium together with the yields on the black soils should be at the level of 40–60% (Hospodarenko *et al*., 2019).

Table 3 shows that the balance of potassium in the crop rotation consists of the same parts as those of phosphorus. The removal of potassium together with the grain on average over two rotation cycles was insignificant (17.2–37.8 kg ha^{-1} of the area under crop rotation over a year) and increased two-fold under the application of the complete mineral fertilizer. The balance of potassium in all variants with the application of potassium fertilizers at the rates of 40–80 kg of active substance ha^{-1} formed positively with the intensity of 111–336%. It was the lowest in the variant $N_{110}P_{60}K_{40}$. A considerable part of potassium returns to the soil with non-marketable products (straw of winter wheat, spring barley, soybean, and leaf-stem mass of corn). This potassium is available to all crops in the crops rotation; therefore potassium fertilizers should be applied under potassium-filling crops on the podzolized black soil. Other scholars noted that a low deficit balance of potassium in field crop rotation is admissible and justified (Lapa and Tsybulko, 2018).

Considering the economic crisis, it is suggested to use the whole or the part of the non-marketable part of the yields as an alternative raw material for fuel. It is necessary to know how the balance of nutrients and soil humus is formed (Hospodarenko *et al*., 2018). According to Lapa and Tsybulko (2018), in the five-field rotation in the Central Forest-Steppe of Ukraine 24–26% of nitrogen, 22–24% of phosphorus, and 33–35% of potassium return together with plant residues into the soil. When 4 t ha^{-1} of non-marketable produce is plowed in the chain soybean-oats-corn, it permits compensation for the expenses of nitrogen on the formation of the yield by 43%, phosphorus by 35%, and potassium by 90% and improves the increase of the crop yielding capacity by 15%.

Calculations have shown that when the non-grain part of the yield was removed from the field, there was a negative nitrogen balance in all variants of the experiment (Table 4). Thus, in the variant $N_{110}P_{60}K_{80}$, the removal of nitrogen was covered only by 76%. Even under the application of nitrogen fertilizers in rotation at the rate of 110 kg ha^{-1} the intensity of nitrogen balance made up 97%. When the non-marketable part of the yield was removed from the field the phosphorus balance was 30–68 kg ha^{-1} depending on the variant except the variant $P_{60}K_{80}$, where the balance was positive – 7 kg ha^{-1}. Under such an approach to the plant growing the rate of phosphorus fertilizers (60 kg ha^{-1}) in the composition of the complete mineral fertilizer should be increased by 25–30%. Potassium balance in the crop rotation when the straw, leaf-stem mass was removed from the field, was drastically negative with the intensity of 32–102% depending on the fertilizing variant. As a result, the removal of a non-marketable part of the yield from the field creates a deficit balance of the main nutrients even in the case when $N_{110}P_{60}K_{80}$ ha^{-1} is applied under crop rotation.

The application of non-marketable crop products of the crop rotation for fertilizing without additional application of mineral fertilizers compensates the expenses of nitrogen on the formation of the yield only by 19%, phosphate and potassium by 34 and 69% correspondingly. In the variant with $N_{110}P_{60}K_{80,}$ these indexes constitute 24, 33, and 71%, respectively.

Hence, the non-marketable products eliminated from the field can increase soil erosion, deteriorate physical properties and decrease organic matter content. These variables can affect the dynamics of water and nutrients, and therefore crop yields.

Table 4. Balance of essential nutrients in the soil during the crop rotation and its intensity when non-marketable part of the yield is removed from the field in the period from 2011 to 2018

Balance item	Variant of the experiment										
	Without fertilizers	N_{55}	N_{110}	$P_{60}K_{80}$	$N_{110}K_{80}$	$N_{110}P_{60}$	$N_{55}P_{30}K_{40}$	$N_{110}P_{60}K_{80}$	$N_{110}P_{30}K_{40}$	$N_{110}P_{60}K_{40}$	$N_{110}P_{30}K_{80}$
Nitrogen											
Supply (kg ha^{-1})	21	81	139	26	142	144	85	150	144	149	145
Removal (kg ha^{-1})	80	121	144	109	156	170	143	196	178	190	181
With main products	64	95	110	87	119	129	111	150	135	144	138
With non-marquetable products	16	26	34	22	37	41	32	48	42	45	43
Balance (kg ha^{-1})	-59	-40	-5	-83	-13	-26	-58	-48	-33	-41	-36
Balance intensity (%)	26	67	97	24	92	85	59	76	81	78	80
Phosphorus											
Supply (kg ha^{-1})	–	–	–	60	–	60	30	60	30	60	30
Removal (kg ha^{-1})	37	56	68	53	73	86	70	99	90	94	88
With main products	21	30	34	31	36	45	38	51	48	49	45
With non-marquetable products	16	26	34	22	37	41	32	48	42	45	43
Balance (kg ha^{-1})	-37	-56	-68	7	-73	-16	-40	-39	-30	-34	-58
Balance intensity (%)	–	–	–	113	–	70	43	61	33	64	34
Potassium											
Supply (kg ha^{-1})	–	–	–	80	80	–	40	80	40	40	80
Removal (kg ha^{-1})	56	78	90	79	105	110	99	132	115	124	121
With main products	17	23	26	24	31	32	29	38	33	36	35
With non-marquetable products	39	55	63	55	74	79	70	94	82	88	87
Balance (kg ha^{-1})	-56	-78	-90	1	-25	-103	-59	-52	-75	-84	-41
Balance intensity (%)	–	–	–	102	76	–	40	61	35	32	66

CONCLUSIONS

In agricultural removal of the nutrients with the grain yield in crop rotation (winter wheat, corn, spring barley, soybean) the largest part is made up of nitrogen (64.4–149.9 kg ha^{-1}), phosphorus – 21,1–51,4 kg ha^{-1} depending on the rates and combinations of mineral fertilizers. From agrochemical and ecological approaches when a non-marketable part of the harvest is left on the field the best balance of nutrients in the crop rotation is formed in the variant $N_{110}P_{60}K_{40,}$ which provides the balanced intensity of nitrogen, phosphorus, and potassium 103, 122, and 111%, respectively. When the non-marketable part of the yield is removed from the field there is a deficit balance of nitrogen, phosphorus, and potassium with the intensity of 76, 76, and 61% even in the variant with the average annual application of $N_{110}P_{60}K_{80}$. The application of non-marketable crop products in the crop rotation for fertilizing in the variant $N_{110}P_{60}K_{80}$ makes it possible to compensate for the losses of nitrogen for the formation of their yield by 24%, phosphorus by 33%, and potassium by 71%.

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Forage quality in a neotropical savanna based on different types of fertilization

Calidad del forraje en una sabana neotropical basada en diferentes tipos de fertilización

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ABSTRACT

The application of different sources of nutrients to the soil with varying degrees of solubility, as well as the use of organic and inorganic fertilizers, can generate different responses in agroecosystems. The objective of this study was to evaluate the effect of different fertilization options on the quality of forage in the conservation-based agroecosystems of neotropical savannas. Both perennial species *Brachiaria dictyoneura* and the legume *Centrosema macrocarpum* are associated with corn cultivation. Four fertilization treatments were evaluated within each cover crop, applying 150 kg ha^{-1} of nitrogen, 150 kg ha^{-1} of P_2O_5, 100 kg ha^{-1} of K_2O, and varying the source of phosphorus, being the treatments distributed as follows: i) phosphoric rock, high dose of phosphorus (100% of P_2O_5 as phosphoric rock), ii) diammonium phosphate, high dose of P (50% of P_2O_5 as phosphoric rock and 50% as diammonium phosphate), iii) biological fertilization, low dose of P (25% of P_2O_5 as phosphoric rock and inoculation with biofertilizer based on native arbuscular mycorrhizal fungi, such as Gigaspora, Scutellospora, Acaulospora, and Glomus), and finally, iv) the unfertilized treatment. The results show that under direct sowing and the use of biological fertilization sources using phosphoric rock as a source of P, similar and even higher levels of raw protein can be reached than when using fertilization with soluble sources such as the diammonium phosphate, which induce greater sustainability of the cover biomass, being an alternative in the management of this types of agroecosystems.

Keywords:
Agroecosystems
Biomass
Cover crop
Nitrogen fixation

RESUMEN

La aplicación de distintas fuentes de nutrientes al suelo, con variados grados de solubilidad, así como el uso de fertilizantes de tipo orgánico e inorgánico, pueden generar diferentes respuestas en los agroecosistemas. El objetivo de este estudio fue evaluar el efecto de diferentes opciones de fertilización sobre la calidad del forraje en el manejo conservacionista de sabanas neotropicales. Las especies introducidas fueron *Brachiaria dictyoneura* y la leguminosa *Centrosema macrocarpum*, ambas perennes, asociadas al cultivo de maíz. Dentro de cada cobertura se evaluaron cuatro tratamientos de fertilización, aplicando 150 kg ha^{-1} de nitrógeno, 150 kg ha^{-1} de P_2O_5, 100 kg ha^{-1} de K_2O, y variando la fuente de fósforo, quedando los tratamientos distribuidos de la siguiente forma: i) roca fosfórica, dosis alta de fósforo (100% del P_2O_5 como roca fosfórica), ii) fosfato diamónico, dosis alta de P (50% del P_2O_5 como roca fosfórica y 50% como fosfato diamónico , iii) fertilización biológica, dosis baja de P (25% del P_2O_5 como roca fosfórica e inoculación con biofertilizante a base de hongos micorrízicos arbusculares nativos, como Gigaspora, Scutellospora, Acaulospora y Glomus) y por último, iv) el tratamiento sin fertilizar. Los resultados permiten concluir que bajo siembra directa y uso de fuentes de fertilización biológica empleando roca fosfórica como fuente de P, se pueden alcanzar niveles de proteína cruda similares e incluso mayores, que al utilizar fertilización con fuentes solubles como el fosfato diamonio, por lo cual se induce a una mayor sostenibilidad de la biomasa de las coberturas, siendo una alternativa en el manejo de este tipo de agroecosistemas.

Palabras clave:
Agroecosistemas
Biomasa
Cultivos de coberturas
Fijación de nitrógeno

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Well-drained savanna production systems in Venezuela are acidic, coarse-textured, with a low capacity to retain water and nutrients, especially phosphorus and calcium. The characteristic production systems of the zone are based on extensive cattle raising on low-quality natural pastures (Ramírez-Iglesias *et al*., 2021).

Agroecological technologies, such as cover crops have been tested to provide alternative soil uses for low-income farmers (Gwenzi, 2021). Such alternatives aim at augmenting the biomass of micro-and macrobiota in soils and its application in agroecosystems (Nascimento *et al*., 2021).

The inclusion of cover crops in agricultural or pastoral rotation systems is a very effective tool for the integrated management of weeds, being a key factor in the minimization of agrochemical applications (Campos *et al*., 2021; Watters, 2021). This occurs mainly because of the effect they have in lowering the weed pressure for the following crop. Besides, they have a leading role in soil conservation, since they get cover crops throughout the year, significantly reducing soil losses due to erosion. These benefits derived from the inclusion of cover crops favor the sustainability of productive systems and can be considered part of a gradual transition to agroecological production systems (Campos *et al*., 2021).

The wide use of cover crop mixes still requires many adjustments both in technical parameters and in agronomic management, in order to achieve a high balanced biomass mix (Ramírez-Iglesias *et al*., 2020). In general, this technique succeeds in increasing the fertility of the surface soil in the short term, due to the beneficial contribution of the roots, reducing erosion to a minimum, in conservation agriculture systems with total coverage, compared to studies in non-coverage and tillage systems (Albarracín-Zaidiza *et al*., 2019; Correa *et al.,* 2020). This practice significantly reduces the use of industrial chemical synthesis fertilizers. Thus, in addition to the direct benefits on the soil, others of an economic, social, and environmental nature point towards a more sustainable agriculture (Albarracín-Zaidiza *et al*., 2019; Francisquini *et al*., 2020).

The use of phosphoric rock (RF) in acid soils has also been widely reported as a beneficial option. The principle of this benefit lies in the variable dissolution rate of RF, which depends on the concentration of protons (H^+) and the reaction products, Ca^{2+} and $H_2PO_4^-$ in solution, around the RF granules (Beura *et al*., 2021). Thus, the rate of RF dissolution increases as the acidity increases and the concentrations of P and Ca^{+2} in the soil decreases (Niswati *et al*., 2021). In this sense, Alewell *et al.* (2020) pointed out that there is a strong influence on the size of the Ca sink in the RF dissolution, therefore, a high Ca extraction by the crop could maintain a low level of Ca^{+2} in the soil solution, which would allow the RF dissolution to continue.

It has further been proved that the association of grasses and leguminous plants provides greater efficiency to the soil for RF dissolution since it is associated with the dependence of biological fixation of N and the greater Ca absorption in leguminous plants, in relation to grasses. The latter contributes to an excess of accumulated cations and greater efficiency in the use and dissolution of RF (Maiquetía *et al*., 2020), and therefore to greater use by the crop.

The use of cover crops such as grasses and legumes is presented as a key to livestock with ruminants (Nascimento *et al.,* 2021) because it generates multiple benefits including the conservation of soil moisture, and legumes to consider the symbiosis that occurs in its rhizosphere (Chippano *et al*., 2021; Moura *et al*., 2020).

The fixation of biological N_2, originating in the Rhizobium-Leguminosae association, is enriched in otherwise N deficient soils (Hernández *et al*., 2020), which favors the application of ATP by bacteria to generate a capitation process by the nitrogenase enzyme complex, reducing N_2 to NH_3, and eventually to NH_4^+, which is absorbed in its soluble form by roots and transformed to organic N. A symbiotic relation occurs when bacteria provide plants with the needed NH_4^+, while the plants generate radical carbohydrate-rich exudates, which can be captured by bacteria in the formation of microbial biomass (Chippano *et al*., 2020).

Ramírez-Iglesias *et al.* (2021) observed a mutualistic association between roots and arbuscular mycorrhiza, in which plants form nutrient-poor savanna, shape and stabilize soils, improving fertility and the formation of

aggregates from mechanical and chemical activities. Chippano *et al*. (2021) support this since these authors described mycelial webs catching and compacting primary soil particles to greater depths, improving the caption of PO_4^{-3} and N mineralization together with microbial activity. The forage improvements related to the productivity of agroecosystems were elucidated in improved forage and favor a balance of N in the agroecosystem that is reflected in its productivity (Hernández *et al*., 2020; Maiquetía *et al*., 2020).

This variability in the amount of forage is essential to estimate it at different times of the year, which consequently will allow predicting, among others, indicators associated with the management of grazing. These indicators analyzed systematically will allow minimizing the risks that lead to degradation conditions of the pasture ecosystem in the production unit (over and under grazing), associated mainly with decisions of grazing time and rest of the pasture and animal load (Ramírez-Iglesias *et al.,* 2021).

In this context, this research aimed to evaluate the dynamics of aerial biomass (green, dry, and stem), leaf/stem relationships, and crude protein content under different types of phosphoric fertilization in a cereal-livestock conservation management system.

MATERIALS AND METHODS

Characteristics of the study area

The research was developed in the Experimental Station "La Iguana", which belongs to the National Experimental University Simón Rodríguez located at 8°25' N; 65°25' W and 80-120 masl, in the well-drained savannas of Venezuela, corresponding to the Tropical Dry Forest, where the climate is seasonal with two climatic periods; a dry one from November to May, and a rainy one from June to October.

Establishment and management of the trial

Before this work was carried out, a conservationist management was established in the area for the development of cereal-livestock production units. For this purpose, two types of perennial coverings were introduced in the agroecosystem: a leguminous, *Centrosema macrocarpum* (Cm), and a grass, *Brachiaria dictyoneura* (Bd). The natural vegetation of the savanna was considered as the control treatment. Both covers were established for two years and a first cut was made before the direct sowing of the corn.

Centrosema macrocarpum is a perennial herbaceous legume with an ascending, vigorous, and curly habit. It is adapted to various types of soils, such as acidic and medium fertility soils, but well-drained, which shows resistance to drought. It has high contents of minerals and proteins. It is desirable for livestock and resists intensive grazing (Akdeniz *et al.,* 2019; Bondaruk *et al.,* 2020; Chippano *et al.,* 2020). *Brachiaria dictyoneura* is a perennial cespitose grass with semi-erect to prostrate and dense growth, pubescent, with long and green lanceolate leaves and dark purple coloring on its edges, and it grows to heights of 0.4 and 0.9 m. Due to the aggressiveness of its growth, it has been recommended to sow it alone and not in association (Correa *et al.,* 2020).

The establishment of the covers was between 2002 and 2004, for which the area was deforested for the trial. Then, the preparation of land was carried out using two cross passes of a light harrow. The replanting of the plots under the introduced covers was conducted as well as a sampling of soil and vegetation for initial characterization. They were later fertilized with 300 kg ha^{-1} of phosphoric rock, applied by broadcasting, and incorporated with a pass of harrow, all in 14 days. Six days later, the introduced covers were sown with 4 kg ha^{-1} for the grass (*Brachiaria dictyoneura*) and 3 kg ha^{-1} for the legume (*Centrosema macrocarpum*), and two years later the sampling was carried out to evaluate the effect of the covers on the soil properties.

During the period 2005-2006, the first cycle of corn-cattle was made, starting with the demarcation of the fertilization management plots (18x350 m), with sampling before the cut of covers. Then, a rotary pass for the uniformization of covers was carried out. Control weed was performed using 2 L ha^{-1} of Paraquat. Later, the direct sowing of hybrid corn variety Imeca 3005 treated with Thiodicarb was performed (60000 plants ha^{-1}).

A conservationist approach to fertilization was used in sandy soils, with basic fertilization of 40 - 100 - 40 kg ha^{-1} $N\text{-}P_2O_5\text{-}K_2O$, in the form of Urea - Diammonium Phosphate

- Potassium Chloride; a first re-sowing with 30 kg ha^{-1} N - 40 kg ha^{-1} K_2O, 23 days after sowing with Urea - Potassium Chloride, and a second re-sowing with 30 kg ha^{-1} N at 41 days after sowing, as Urea. Then a sampling was carried out during the corn flowering period and later the corn was harvested.

Once the cereal was harvested at the beginning of the dry season, an intensive grazing was made with cattle equivalent to 1.04 UA ha^{-1} for 110 days so that they could feed with the covers and corn stubble, culminating with a sampling after the grazing.

Experimental design

A completely randomized design with a 2x4 factorial arrangement (2 types of cover crop and 4 types of fertilization) and 12 repetitions distributed in three sampling units were used in each treatment. Due to the need to use large experimental units to evaluate the effect of management on the plant, animal, and soil properties (Turner and Carpenter, 1999, Ramírez-Iglesias *et al.*, 2020), plots of approximately 2.6 ha (75x350m) were used for each cover crop (Bd and Cm).

Within each cover crop, fertilization types were placed in 18x350 m plots and within each fertilization type, sampling units were 900 m^2 (15x60 m). The dimensions, orientation, and the number of samples to be taken in each of the experimental plots were determined based on a previous study of spatial variability (Bravo *et al.*, 2004; Lozano *et al.,* 2004). The experiment was performed in the dry season since part of the management includes the harvest of corn and the introduction of livestock to take advantage of corn residues, as part of the management of grazing.

Within each cover, four types of fertilization were established, with basic fertilization with 150 kg ha^{-1} of N – 150 kg ha^{-1} P_2O_5 – 100 kg ha^{-1} K_2O, and variation of the phosphorus source, being the treatments as follows Io: control without fertilization (0 N - 0 P_2O_5 - 0 K_2O); RF: high dose of phosphorus (100% of P_2O_5 as phosphate rock); IR: high dose of P (50% of P_2O_5 as RF and 50% as diammonium phosphate, FDA); FB: low dose of P (25% of P_2O_5 as RF and inoculation with biofertilizer based on native arbuscular mycorrhizal fungi).

Determination of biomass and forage quality

For the quantification of the total aerial biomass of the forage, a ring of 50 cm in diameter was used, taking the plant material located on the surface of the soil that was enclosed in the ring. This procedure was carried out by throwing the ring 6 times randomly, in five punctual moments during the grazing period (n=24), at 0, 41, 60, 81, and110 days after the animals were introduced (DAIA) in each paddock of the cover crop and fertilization treatments. The total number of samples in the analysis for the five sampling moments was n=120.

The material was weighed and separated into green, dry, and stem biomass fractions, then was dried in an oven at 40 °C for 72 h (Ciavatta *et al.*, 1989), and processed in a Wiley mill for further analysis. Likewise, the green: dry and leaf: stem ratios were performed.

The crude protein was determined for each sample (CP) through wet digestion with H_2SO_4 and H_2O_2 and the CP was determined, from the % of N (Bremmer, 1982; Ciavatta *et al.*, 1989).

Statistical analysis

The results obtained in the different stages were subjected to descriptive exploratory analysis, verification of statistical assumptions, and the detection and elimination of anomalous values, per cover treatment (green and dry biomass, and leaf), soil (0-15 and 15-30 cm) fertilization (RF, IR, FB, Io), and seasons (5-moment sampling).

The normality of data was evaluated with descriptive analysis of mode, mean, asymmetry, and kurtosis. Data distribution was illustrated as variance (S2), standard deviation (SD), variance coefficient (CV), and maximum and minimum values.

The interactions between the covers of *Brachiaria dyctioneura*, *Centrosema macrocarpum,* and natural savanna exposed vegetation to four fertilization levels: i) phosphoric rock (RF), high dose of phosphorus (100% of P_2O_5 as phosphoric rock), ii) diammonium phosphate (IR), high dose of P (50% of P_2O_5 as RF and 50% as FDA), iii) biological fertilization (FB), low dose of P (25% of P_2O_5 as RF and inoculation with biofertilizer based on native arbuscular mycorrhizal fungi, such as *Gigaspora, Scutellospora, Acaulospora,* and *Glomus*), and finally, iv) the unfertilized treatment (Io) and the effects of grazing
The linear statistical model was applied as follows:

$$Y\ ijklm\ldots v=\mu+ Ci + Fj+Gk+El+ (C \times F)ij+(C \times G)ik+(C \times E)il+(F \times G)jk+ (F \times E)jk + (C \times F \times G \times E)\ \varepsilon ijkl$$

Where:

μ= population mean

Yijkl= response of the variable in the i-th treatment and the l-th repetition.

Cijkl= effect of cover crops in the i-th treatment and the l-th repetition

Fijkl= effect of fertilization treatments (IR, Io, FB, RF) in the i-th treatment and the l-th repetition. l-th replicate

Gijkl= effect of livestock on the j-th treatment and the l-th repetition

Eijkl= epoch (sampling times during maize growth) at the i-th treatment and the l-th repetition

εijkl= experimental error in the i-th treatment and the l-th repetition.

The ANOVA and comparison of means among cover crops were carried out, times, and types of fertilization with Duncan's test (Duncan, 1974) at a level of significance of $P<0.05$. The statistical program InfoStat was used for the analyses (Di Rienzo *et al*., 2020).

RESULTS AND DISCUSSION

Effects of phosphorus fertilization on the dynamics of biomass fractions of cover crops during the grazing cycle

The results obtained in the research, refer to the effects of the type of phosphoric fertilization on the production of the green leaf biomass (GLB), dry leaf biomass (DLB), and stem biomass (SB) fractions in corn-livestock agroecosystems, which are shown in Figure 1.

On average, Bd in GLB was significantly higher ($P<0.05$) when IR fertilization was used (1416 kg ha^{-1}), followed by FB (1378 kg ha^{-1}), RF (966 kg ha^{-1}), and finally without Io fertilization (621 kg ha^{-1}). In this same species, significant changes were also observed when evaluating the fractions of dry leaf biomass and stem biomass, due to the effect of fertilization, appearing in the following descending order FB>RF ≥ IR>Io.

It can be observed that the GLB of the grass decreased in the first 80 days of grazing in all fertilizations, exhausting this fraction in both IR and Io. Except for RF, all fertilization operations resulted in a recovery of GLB production at the end of the grazing cycle.

This trend is possibly related to better soil moisture preservation under this type of fertilization, which is possibly associated with nutrient availability and leaf regeneration in the short term. In this regard, Niswati *et al*. (2021) mentioned that the use of rock in acidic soils improves the yield of plant biomass, as well as the availability of phosphorus such as phosphates and orthophosphates since it promotes the creation of a microclimate in the soil that favors processes of chemical transfer from the soil to the plant and foliar development.

It is important to highlight that the fractions of the grass plants under the three types of phosphorus fertilization plus the control treatment, showed a higher GLB, especially in the treatment where an association with mycorrhizal fungi was established. These findings support the thesis that the FB treatment provided cattle with a greater amount of green leaves during grazing time.

Regarding the DLB, both in IR and RF, this fraction decreased until 40 days, and then there was an increase, which in the case of IR showed to be sustained until the end of the cycle, while in RF just decreased to 80 days and then increased. The DLB of the grass in the FB treatment had similar behavior to the Io treatment, that is when the corn and the grass are not fertilized.

When comparing the dynamics of GLB and grass DLB (Bd), it was observed that as the drought progressed in the grazing cycle, part of the GLB fraction was transformed into DLB. This experiment was carried out during the dry season when the cattle feeding resource is scarcer.

At that stage, the process of photosynthesis and respiration can be affected, and, therefore, the production of green leaves was limited. Analyses conducted on similar crops (Bondaruk *et al*., 2020; Ojo *et al*., 2020) indicated that the leaf and its characteristic features are important in carbon assimilation, water relations, and the energy balance of the plant.

Concerning the SB, the dynamics presented few variations in time, especially in IR and Io, probably because that fraction of the plant is less palatable and less digestible (Francisquini *et al*., 2020; Ramírez-Iglesias *et al*., 2020).

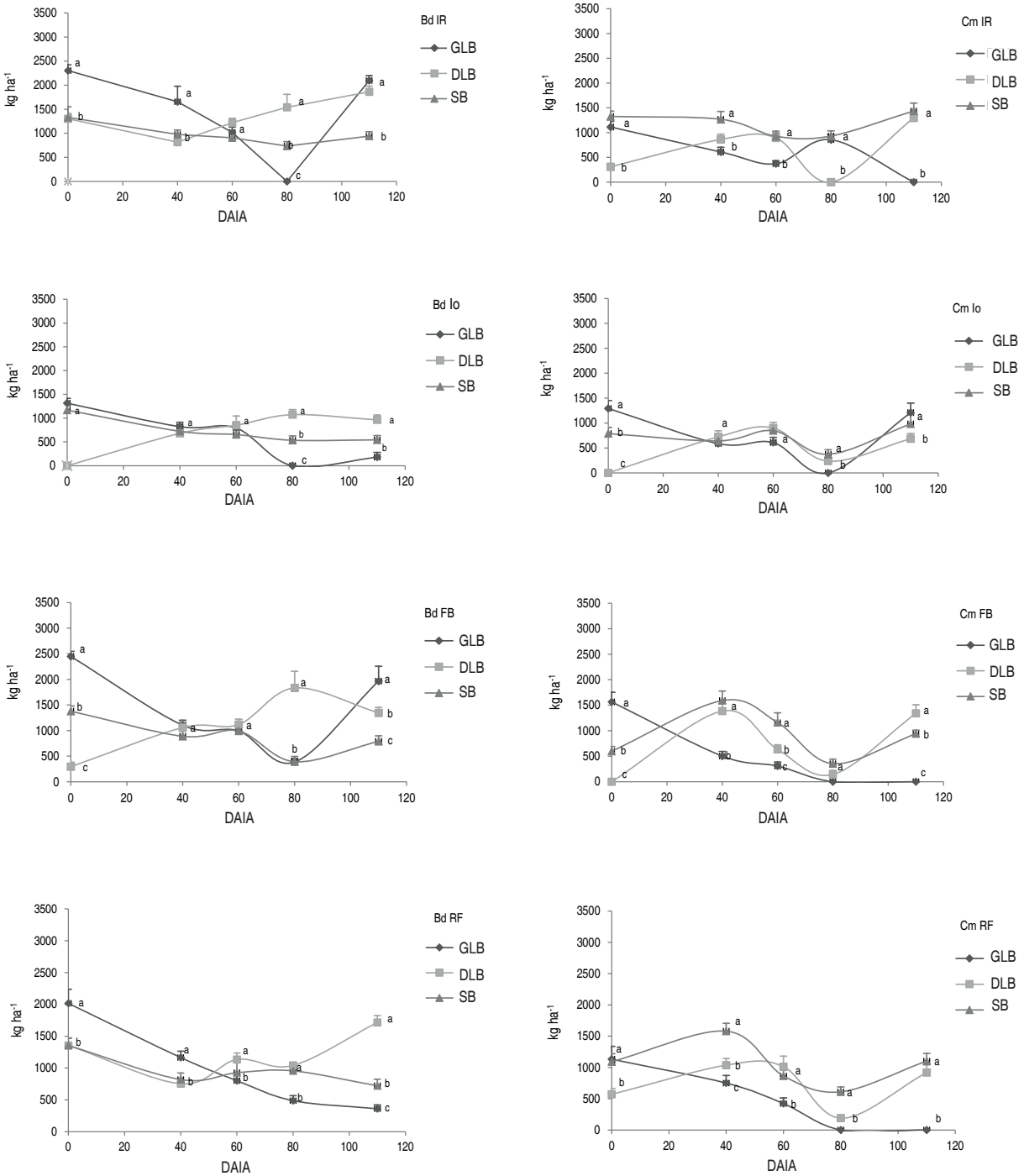

Figure 1. Dynamics of the effect of phosphorus fertilization on the total aerial biomass fractions of *Brachiaria dyctioneura* (Bd) and *Centrosema macrocarpum* (Cm) during grazing. IR: high dose of P (50% of P_2O_5 as RF and 50% as FDA); Io: control without fertilization (0 N - 0 P_2O_5 - 0 K_2O); FB: low dose of P (25% of P_2O_5 as RF and inoculation with biofertilizer based on native arbuscular mycorrhizal fungi; RF: high dose of phosphorus (100% of P_2O_5 as phosphate rock). GLB: green leaf biomass; DLB: dry leaf biomass; SB: stem biomass. DAIA: days after the introduction of the animals (different letters indicate statistically significant differences in each moment of sampling, bars indicate standard deviation. Duncan $P<0.05$, n=24).

In the case of legumes, the biomass production of the fractions was lower, especially in GLB. A decrease was experimented until disappearance after 80 days, except for the treatment that used high soluble P sources (IR), where GLB remained longer until completely disappearing 110 days later. The green leaf fraction recovered at the end of the cycle, only under the control treatment (Io). Studies in this type of agroecosystem (Chippano *et al.,* 2020) indicate that the contents of neutral detergent fiber (NDF) may relate to the stem disappearance of the green fraction since these contents tend to be lower in the green leaves, as it was reported in other studies (Mora *et al*., 2019).

In relation to SB, it can be said that its contribution was significant, increasing throughout the grazing cycle because the cattle did not consume the stems due to their low palatability and lower digestibility (Mora *et al*., 2019; Ramírez-Iglesias *et al.,* 2020), so its behavior over time was similar to that presented by the dry leaf biomass. As previously mentioned, this behavior is in accordance with Cm, since despite being an herbaceous legume, its stem has woody characteristics (Bondaruk *et al*., 2020; Campos *et al*., 2021; Hernández *et al.,* 2020). In Cm, GLB was lower compared to DLB and SB, causing higher consumption by cattle, since this indicator is a measure of hemicellulose, cellulose, and lignin, which represents the entire fibrous component of the forage.

The production of both grass and legume fractions is influenced by the type of phosphorus fertilization, which favors the production of GLB in the case of grass by the FB, while Cm tends to be more persistent over time in the case of IR. Under these types of cover-fertilization, the plant/animal relationship during grazing is likely improved, making it more persistent and sustainable in the long term.

In intensive grazing, the resprouts emerge depending on the severity of the grazing (Bondaruk *et al.* 2020). The proportion of young leaves left in the remnants of recently grazed plants is what makes the photosynthetic potential of the pasture possible (Chippano *et al.,* 2021; Nascimento *et al.,* 2021), which in turn is related to the speed of recovery of reserve carbohydrates (de Melo Lisboa *et al.,* 2021).

Research in production systems with corn (Mora *et al.,* 2019; Lozano *et al*., 2004) indicate that when using different organo-mineral mixtures for crop fertilization green biomass can be obtained. Consequently, it can be a congruent alternative with the sustainable management of the soil in similar edaphoclimatic conditions.

Dynamics of the leaf/stem ratio (L/S) of the covers under different types of phosphorus fertilization

Figure 2 shows the variations in L/S ratios overtime during grazing, according to the types of cover and fertilization applied.

At the end of the grazing stage, the order of the L/S ratio in the treatments was in the case of the grass: FB>IR>RF>Io, indicating the important recovery of leaf biomass over the stem biomass of the grass when biofertilizers or FDA mixed with RF were used (Figure 2A). Subsequently, the dynamics passed without significant differences between treatments. However, after 80 days, there was a significant increase in the proportion of L/S, in the use of biofertilizers mixed with 25% of P as phosphate rock FB, which was maintained until the end of grazing.

The tendency to maintain the L/S dynamics in the long term under FB could be associated as it is mentioned by Moura *et al*. (2020) with the presence of some bacterial genera such as *Stenotrophomonas* sp, *Burkholderia* sp, *Pseudomonas* sp, *Rhizobium* sp, among others, that participate in the solubilization of phosphates since they tend to increase the presence of phosphorus ions when being hydrolyzed with enzymes as the phytases, facilitating the mobility of this element in the soil and transform it into a compound available immediately for the plant (Chippano *et al*., 2020; Hernández *et al*., 2020). This management strategy is to be considered, especially in systems where there are important limitations of P in the soil.

For the legume Cm, no significant differences in L/S dynamics were observed in the different fertilization treatments during grazing, although the trend was to slowly decrease until 80 days, with a slight increase at the end of that stage (Figure 2B). This behavior may be because animals tend to select more leaves for consumption, leaving aside the thicker and more lignified stem, since cattle move in a horizontal plane, but select their diet in a vertical plane (Lebbink *et al.,* 2018).

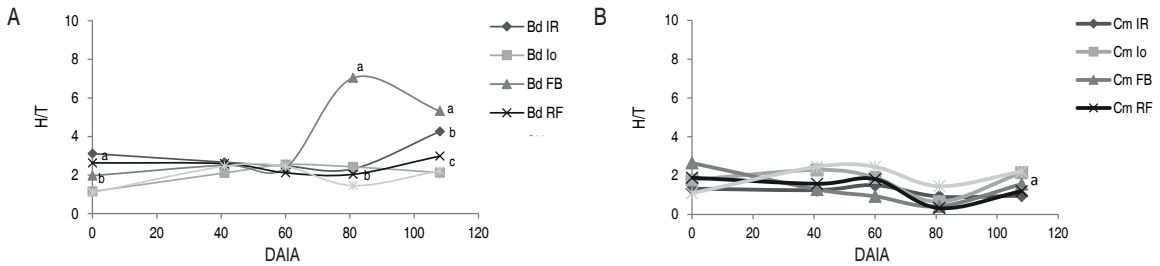

Figure 2. Dynamics of the leaf/stem ratio during grazing in A) the *Brachiaria dictyoneura* grass and B) the *Centrosema macrocarpum* legume under different sources of fertilization during cover growth. IR: high dose of P (50% of P_2O_5 as RF and 50% as FDA); Io: control without fertilization (0 N - 0 P_2O_5 - 0 K_2O); FB: low dose of P (25% of P_2O_5 as RF and inoculation with biofertilizer based on native arbuscular mycorrhizal fungi); RF: high dose of phosphorus (100% of P_2O_5 as RF); DAIA: days after animals are introduced. Bars indicate standard deviation (different letters show statistically significant differences between fertilization treatments for each moment of sampling, Duncan $P<0.05$, n=24).

The L/S ratio was lower for Cm compared with Bd because of the high proportion of stems in Cm.

Tapia *et al.* (2019) regarding productivity and quality of forage, indicate that the L/S ratio is an index that allows expressing the quality of forage. In general, ratios>1, indicate that there were a greater proportion of leaves than stems, which could indicate that the plants are young or are sprouting from a cut, and therefore, there is greater availability of higher quality food for the animal.

Related studies in this type of agroecosystems (Morantes *et al.,* 2010; Morantes *et al.,* 2018) where grasses such as *Brachiarias*, *Andropogon*, and *Panicum* were used, report lower L/S values (between 0.8 and 0.6) for dry and rainy seasons in savanna ecosystems compared to those found in the present study, indicating the low quality of native savanna pastures.

Research on similar agroecosystems for milk production (Ramírez-Iglesias *et al.,* 2017), showed a decrease in L/S in cattle production systems, reporting values between 1.7 and 2 in pastures with better forage quality, where it is indicated that L/S dynamics are related to management, type of grazing, and a lesser extent, to the activation of growth or regrowth of the pasture as a result of rainfall, therefore, an adequate combination of management (cover-fertilization and grazing) in the evaluated agroecosystem will allow improving the relationships between soil-plant and animal components in the long term in a critical time for livestock such as the dry season.

Effects of different sources of phosphorus fertilization on the dynamics of the crude protein in the biomass fractions of the covers

The CP content in the GLB fraction in Bd, presented significant statistical differences ($P<0.05$) when the animals were introduced to grazing (Figure 3), showing the following trend: IR>FB>RF>Io, and then decrease their levels at the end of the cycle, except for the treatment where only RF was used, which maintained more stable percentages over time, presenting the highest values at 60 and 80 days. At the end of the cycle, the effect of the type of fertilization was lost, therefore, no significant differences were observed between treatments.

CP contents in the BD at the beginning of the cycle were higher and decreased as grazing progressed in Bd, reaching the lowest values at 60 and 80 days, except in the treatment in which only phosphate rock was used, where even levels of 12% CP were reached at the end of grazing (110 days). The dynamics suggest that under this type of slow-release P fertilization, the senescent material will provide more N in less time, compared to the dry leaf biomass under another type of fertilization. This considered, and depending on the C content, the C/N ratios favor a greater and faster decomposition in RF (100% phosphate rock), which will influence the cycling of nutrients within the agroecosystem.

The CP percentages obtained for DLB fractions for Bd were similar to those reported in conservationist systems, where dry leaves of grasses were evaluated, finding contents of up to 9.7% CP (Lebbink *et al.,* 2018; R. Vera-Infanzón and

Ramírez-Restrepo, 2020). Concerning other works carried out in Venezuela, specifically in the studied zone, values of 10.5% of CP, in foliar weave have been indicated, not specifying if it was in green or dry biomass (Berroterán, 2000).

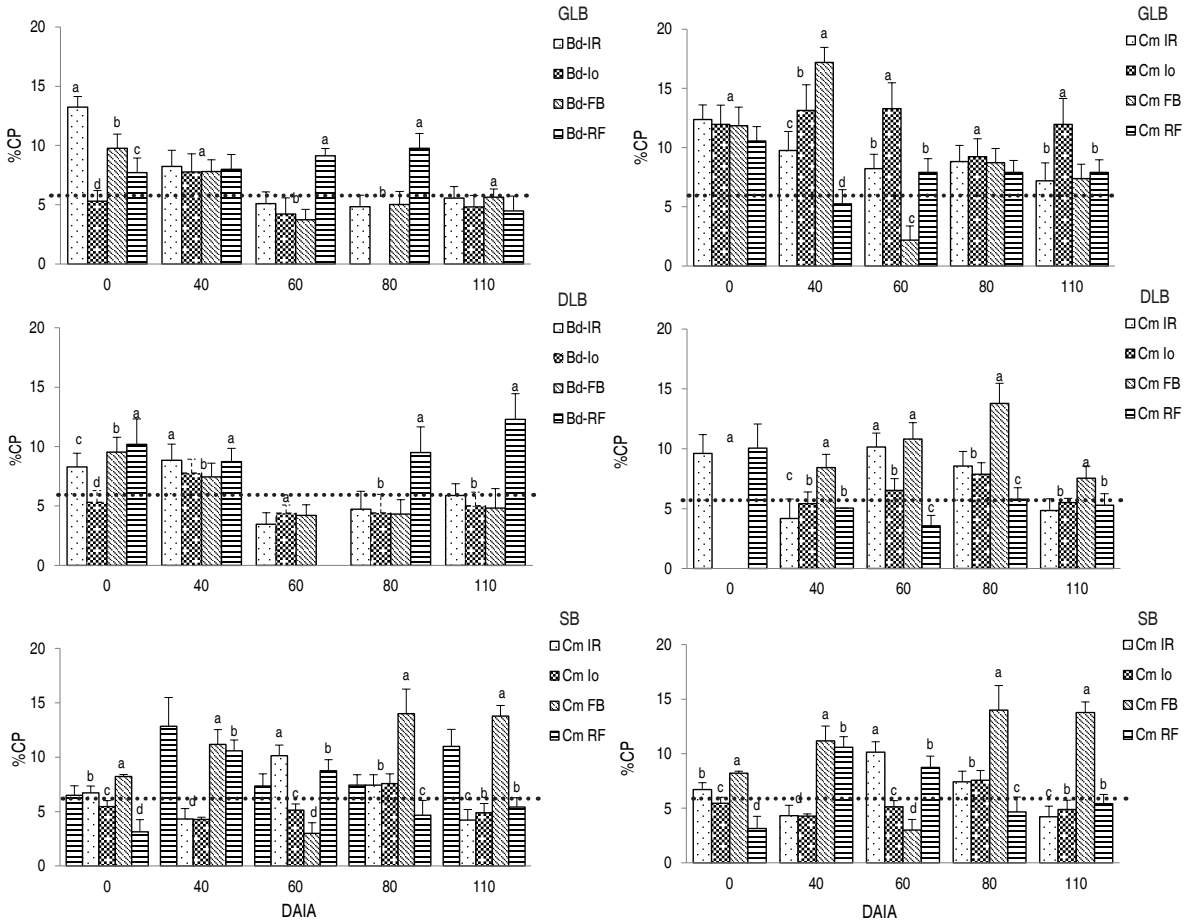

Figure 3. Dynamics of raw protein concentration, in the fractions of green leaf biomass (GLB), dry leaf biomass (DLB), and stem biomass (SB) in *Brachiaria dictyoneura* and *Centrosema macrocarpum* during grazing and under different fertilization sources. IR: high dose of P (50% of P_2O_5 as RF and 50% as FDA); Io: control without fertilization (0 N - 0 P_2O_5 - 0 K_2O); FB: low dose of P (25% of P_2O_5 as RF and inoculation with biofertilizer based on native arbuscular mycorrhizal fungi); RF: high dose of phosphorus (100% of P_2O_5 as phosphate rock) DAIA: days after the animals are introduced. Bars indicate standard deviation. The dotted line indicates the critical value of CP 7% for cattle feeding (Herrera *et al*., 2008). (Different letters show statistically significant differences between fertilization treatments for each moment of sampling, Duncan $P<0.05$, n=24).

Bd in SB, CP percentages showed fluctuations in the cycle, starting with lower values in RF, but increasing rapidly after 40 days. While IR, in the treatment where the mixture of FDA and RF was used, this fraction had higher CP contents at the beginning of the studies, but decreased in the same period, exhibiting the lowest levels among all the treatments. In the treatment with the use of biofertilizers, the % CP was presented above the critical values during the first 40 days.

It is important to mention that in this fraction the highest CP contents were reached in comparison with GLB and DLB. It is possible that the high N contents in the SB could be due to the continuous defoliation during the process, which could have promoted the translocation of N to that area, to guarantee the necessary reserves for the next regrowth (Chippano *et al.,* 2020; Depablos and Ordóñez, 2009).

In similar works, under conservationist systems and the use of cover crops, Akdeniz *et al.* (2019); Morantes *et al.* (2010), and Ramírez-Iglesias *et al.* (2020) reported similar values to those obtained in this research on stems for agricultural systems.

In the case of Cm, the averages of %CP in the GLB during grazing showed the following pattern: RF>IR>FB>Io. At the time the animals entered grazing, no significant differences were observed between fertilization treatments. However, at 40 days, the use of FB and RF produced the highest values of crude protein in the GLB.

In Cm, the CP content in the DLB did not present differences between IR and RF treatments at the beginning of grazing. Later, and as the cycle advanced in the middle of the dry season, the CP decreased below the critical levels for grazing, except for the DLB of the treatment with BS. The positive effect of the use of biofertilizers with RF was also observed in the stem biomass.

In the legume, the CP content was low in Io, while in the other treatments it showed a tendency to increase, especially at the end of the grazing cycle, observing a greater increase in the GLB, which could be associated with the fact that there was greater availability of N in the soil, improving the quality of the covers under the indicated fertilizations, as reported by Mora *et al.* (2019). High crude protein values obtained even without fertilization may be related to the N-fixing condition of Cm.

Investigations related to leguminous grazing (Akdeniz *et al*., 2019; de Melo Lisboa *et al*., 2021; Mora *et al*., 2019), point out that CP contents in C3 plants vary between 15 and 25% of dry matter, with the advantage of having a lower rate of decrease in CP content while plant age increases.

Figure 3 depicts different crude protein dynamic patterns in the studied cover crops, Bd and Cm, according to the plant fraction analyzed and the type of fertilizer used. The dotted line indicates the critical value of CP for cattle feeding, which is located at 7% (Berroterán, 2000; Morantes *et al.,* 2010).

In the agroecosystem for both grass and legumes, there were treatments in which the type of fertilization possibly contributed to achieving CP percentages above critical levels (7%) in a more sustained way during the cycle (Depablos and Ordóñez, 2009).

In the case of legumes, the capacity to conserve their protein level in the forage despite their senescence, consequently helps to greater use of the dry matter, especially when associations with grasses are established, as the evaluated agroecosystem.

Bd, under a fertilization source of slow solubilization and use of mycorrhizae, achieved a higher production of green leaf biomass, unlike what was observed in Cm, which although showed better nutritional contents, because it presented a lower production of green leaf biomass, this positive effect was possibly masked in the agroecosystem (Mora *et al.,* 2019; Morantes *et al.,* 2010).

The review and analysis of the trials show that in the context of agriculture with a sustainability approach, agroecological practices have evolved as a result of traditional experiences accumulated by individual producers, associations, and territorial development projects, assuming different scales of application and mechanisms (Campos *et al.,* 2021; Francisquini *et al.,* 2020; Watters, 2021), where the combination of cover-fertilization as part of agroecosystem management can improve soil-plant-animal relationships in terms of long-term biomass and nutrient availability.

CONCLUSIONS

Using low solubility fertilizers (phosphoric rock) aids in reaching crude protein contents at similar and higher levels than when using soluble fertilizers (diammonium phosphate), which induces greater sustainability of the cover biomass over time.

Similarly, the application of mycorrhiza in association with Leguminosae improves the absorption of ammonium and nitrate and the transformation to organic N. This process possibly augmented the presence of P solubilizing microorganisms and better absorption of inorganic P by the cover vegetation of pastures and represents an alternative in agroecosystems limited in N and P.

The use of agroecological strategies facilitates food security with an alternative approach, directed to the recovery and conservation of natural resources and proposals for sustainable development.

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Short article

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Spatial variability characterization of acoustic discomfort and zone of admissible noise caused by micro-tractor

Caracterización de la variabilidad espacial del malestar acústico y zona de ruido admisible por microtractor

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ABSTRACT

Keywords:
Acoustic comfort
Occupational health
Spatial variability

Agricultural development requires greater adoption of machinery by producers to avoid damage to the worker's health due to excessive noise. In this scenario, this study aimed to analyze the noise magnitude emitted by a micro-tractor using geostatistics and Statistical Process Control (SPC) in mapping spatial variability to identify healthy zones for workers. The study was carried out at the Federal University of Lavras (UFLA), where noise levels were collected at points distributed in a regular 2.0×2.0 m sample grid around the machine. The spatial dependence of noise was analyzed by adjusting the wave-type semivariogram and interpolating by ordinary kriging and the SPC through individual control charts. It was possible to observe alarming noise values above 85 dB(A) in a radius of up to 6 m around the tractor in operation. The maximum value of 91 dB(A) was obtained from the operator's seat, thus allowing maximum daily exposure of 3.5 h. In addition, it was observed that people located at distances greater than 4 m from the micro-tractor do not need to wear personal protective equipment for an exposure of 8 h of work.

RESUMEN

Palabras clave:
Comodidad acústica
Salud ocupacional
Variabilidad espacial

El desarrollo agrícola exige una mayor adopción de maquinaria por parte de los productores para evitar el daño a la salud de los trabajadores por el exceso de ruido. En este escenario, este estudio presenta análisis utilizando geoestadística y Control Estadístico de Procesos (SPC) en el mapeo de la variabilidad espacial de la magnitud del ruido emitido por un microtractor para identificar zonas saludables para los trabajadores. El estudio se llevó a cabo en la Universidad Federal de Lavras (UFLA), donde los niveles de ruido se recolectaron en puntos distribuidos en una cuadrícula de muestra regular de 2,0×2,0 m alrededor de la máquina. La dependencia espacial del ruido se analizó ajustando el semivariograma de tipo onda e interpolando por kriging ordinario y el SPC a través de cartas de control individuales. Fue posible observar valores de ruido superiores a 85 dB(A) en un radio de hasta 6 m alrededor del tractor en funcionamiento. El valor máximo de 91 dB(A) se obtuvo desde el asiento del operador, permitiendo así una exposición diaria máxima de 3,5 h. Además, se observó que las personas ubicadas a distancias mayores a 4 m del microtractor no necesitan usar equipo de protección personal para una exposición de 8 h de trabajo.

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Studies on the spatial distribution of acoustic discomfort are essential for improving the work environment, especially in evaluating the salubrity which operators and helpers will be subjected to the operation of a micro-tractor whose work rotation is 2400 rpm. Fernandes and Morata (2002) cite that physical agents such as noise, heat, vibration, pressure, radiation, and chemical agents such as smoke, dust, gases, and vapors, are some of the environmental stressors which are found in several workplaces.

Arcoverde *et al.* (2011) emphasize the importance of studies about noise levels in agricultural operations for the real need of adopting prevention to minimize noise effects and improve comfort and safety.

Bistafa (2018) conceptualizes noise as a sound without harmony, in general of a negative connotation, in other words, in most cases it can be classified as an undesirable way sonorously, standing out as one of the occupational risk agents.

Previous studies have evaluated the noise emitted by agricultural machines through geostatistics, but for the adjustment of the semivariogram, these studies used the spherical model (Ferraz *et al.,* 2013; Júnior *et al.,* 2014) and the Gaussian model (Missio *et al.,* 2015; Pimenta *et al.,* 2012; Spadim *et al.,* 2015). However, these mentioned models may not represent the noise behavior correctly, being necessary to adjust these models as a periodic function or sinusoidal function, as the Wave model used by Gonçalves *et al.* (2019).

In addition, the Statistical Process Control (SPC) was another tool used to analyze these data. SPC is a simple tool, and its effectiveness is witnessed by a repetition physically established in industries around the world. Therefore, it is possible to control significant characteristics of the product and the process in real-time, guaranteeing quality levels at a cost supported by the market (Nomelini *et al.,* 2009).

Among the main tools of the SPC, the control graphs stand out. They are among the most used when it comes to monitoring processes. According to Werkema (1995), the interpretation of control graphs is made through non-random patterns. Therefore, this work aimed to illustrate and discuss such patterns based on statistical and probabilistic concepts.

According to Nomelini *et al.* (2009), SPC is a robust methodology developed to effectively control product quality and production processes, using statistics to analyze the process's limitations. SPC application in noise emitted analysis by agricultural machinery is hardly found in scientific research, highlighting the importance of this study.

It is also noted that the mitigation of the noise problem is linked to reducing the intensity of the same source, reducing the exposure time, or using individual protective equipment. Thus, it is essential to know the reality of the machine under study in terms of noise to plan the best strategy to combat the problem.

Given the exposure, the aim of this study was to use geostatistics and Statistical Process Control (SPC) from individual charts to map and characterize the magnitude of the spatial variability of noise emitted by a micro-tractor, to identify healthy zones for workers.

MATERIALS AND METHODS

The experiment was developed at the Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil. In this study, a micro-tractor with a nominal power of 11 kW (15 hp) was evaluated, whose year of manufacture was 2007. Its working rotation is 2400 rpm. The tractor had a coupled trailer, which did not have any driver.

The noise level rating of this agricultural machine was carried out in which the ambient temperature was between -5 and 30 °C and the air velocity was less than 5.0 m s^{-1}. For the measurement of noise propagated by the tractor, a digital decibel meter model dec 460 was used to measure the noise level emission, the description of which is based on a sound pressure level measuring instrument with instantaneous reading and peak reading, automatic scale, and frequency weighting in A and C. There is a measurement of 35 to 130 dB (A) and internal calibrator in terms of the measurement scale.

Readings were taken at an average height of the operator's ear, at points distributed in a regular 2×2 m

sampling grid. Totaling 441 sampling points around the agricultural machine in stationary operation (Figure 1). These samples were later used in descriptive statistics. A spatial coordinate was arbitrated in meters where the central point (0, 0) corresponded to the point where the agricultural machine remained in operation during all collection. The central point (0, 0) corresponded to the operator's seat (Figure 1).

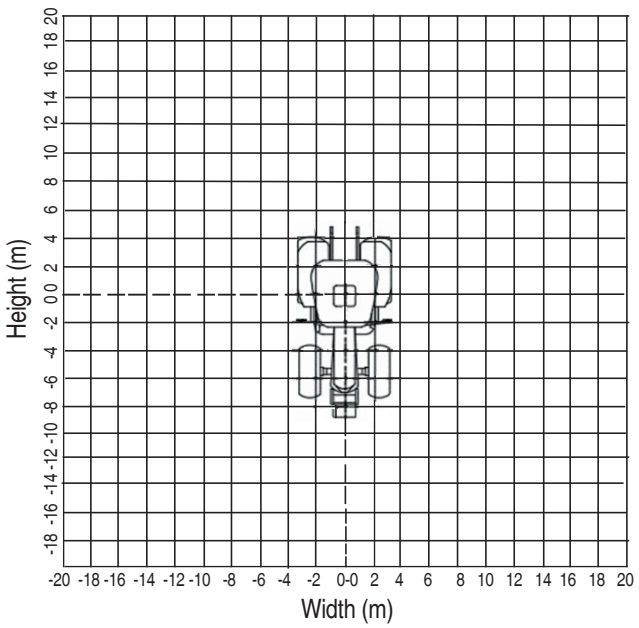

Figure 1. Sampling area of the noise levels emitted by the micro-tractor under study.

The spatial dependence of the noise emitted by the micro-tractor placed in the operation regime was analyzed through adjustment of classic semivariogram by the Ordinary Least Squares (OLS) method and the Wave model. The classic semivariogram was estimated by Equation 1:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} \left[Z(x_i) - Z(x_i + h) \right]^2 \qquad (1)$$

Where N (h) is the number of experimental pairs of observations, $Z(x_i)$ and $Z(x_i+h)$ and separated by a distance h, the semivariogram is represented by the graph $\gamma(h)$ estimated semivariance for distance h. From the adjustment of a mathematical model to the calculated values of $\gamma(h)$, the coefficients of the theoretical model are estimated for the semivariogram called the nugget effect, C_0; threshold, $C_{0+}C_1$ and the reach, a, as described by Vieira *et al.* (1983).

In Equation 2, the theoretical model adjusted to the experimental semivariogram utilized for the research is the Wave model (Mota, 2008; Gonçalves *et al.,* 2019).

$$\gamma(h) = C_1^2 + \frac{a}{h} \sin\left(\frac{h}{a}\right) \qquad (2)$$

Where C_1^2 is the contribution, a is the range, and h is the distance between the points observed.

Equation 2 represents periodic variations, which indicate non-monotonic growth of the semivariance with distance, presenting models with and without threshold (Andriotti, 2004). These non-monotonic structures can have reduced wave amplitudes, be isotropic, and be anisotropic (Carvalho *et al.,* 2004). Another analysis that must be carried out is based on the relationship between theoretical and practical scope. According to Chilès and Delfiner (2012), the practical range of the Wave model is reached when the h is approximately equal to 4.5a.

For the geostatistical analysis, R was used as a statistical computer system with GeoR package (Ribeiro Júnior and Diggle, 2001). SURFER 15.2 software was used to plot the maps. The values recommended by

ABNT (2021) were used for the evaluation of admissible levels. NR 15 norm defines a time limit for workers to be exposed to continuous or intermittent noise that will not harm their health.

In addition to the geostatistical analysis, the noise data emitted by this micro-tractor was evaluated through process control charts (SPC). Control charts also present complementary criteria for quality analysis. Including a sequence of points occurrence, upward downward trends, shifting in the process cyclic patterns, points plotted close to the control limits and clustering around the mainline (Szekut *et al.,* 2018). Civardi (2017) suggested a sequence of steps to analyze the control charts: build experimental control limits after data collection according to the sampling plan; verify that all points are within the control limits and no random configuration is being performed. If these two conditions are met, it can be said that the process is under statistical control, and the next step is to assess the capacity of the process; if there are points outside the control limits and/or some non-random configuration, it may conclude that the process is out of the state of statistical control. In this case, special reasons that caused each point should be identified.

For the composition of individual letters in the SPC, the Minitab 19 software was used. Because of this, it was separated into five stages consecutively. Such stages were formed to facilitate the analysis of the data obtained in the sample grid. From these data, stage 1 was developed from radius 0 to 4 m; stage 2 was created from radius 4 to 8 m, stage 3 was set from radius 8 to 12 m, stage 4 was made from radius 12 to 16 m and the stage 5 consisted of a radius of 16 to 20 m.

RESULTS AND DISCUSSION

The descriptive statistics of the noise emitted by the studied tractor are shown in Table 1. Analyzing the minimum and maximum limits, the coefficient of variation, and the average noise, it was possible to observe a variation in the data. However, this analysis does not demonstrate the spacial position of the lowest and highest values of noise emitted by the tractor, therefore geostatistical studies are required.

The geostatistical analysis of the noise levels emitted by the micro-tractor in the operating regime can be noted in Table 2. Thus, it is observed that the semivariogram (Table 2; Figure 2) and its parameters (nugget effect, "C_0"; contribution, "C_1"; sill, "$C_0 + C_1$"; and range, "a") were obtained by the Ordinary Least Squares method (OLS - ordinary least square) and by the Wave model (Figure 2).

By analyzing Figure 2 and also observing Table 2, the practical range of the spatial distribution of the noise emitted by this machine was 13.94 m, which implies that to this distance, the variable under study is influenced through space. It is observed in Figure 3 that the alarming values of the noise emitted by the micro-tractor, above 85 dB (A), for daily exposure of 8 h (ABNT, 2021) are seen, in general, up to 6 m of distance around the tractor in operation regime. Note that in the center of the map

Table 1. Descriptive statistics of noise (dB) emitted by the studied tractor.

Min	Max	X	Md	SD	Var	CV	K	Asymmetry
59.0	91.20	70.50	69.50	5.418	29.357	0.0770	1.522	1.038

Min – Minimum value of the variable; Max – Maximum value of the variable; X- Average; Md – Median; SD – Standard deviation; Var – Variance; CV – Coefficient of variation; K – Coefficient of kurtosis

Table 2. Methods, models, and parameters estimated the experimental semivariogram to the noise level emitted by a tractor.

C_0	C_1	C_0+C_1	a	a'	ME	SD_{ME}	RE	SD_{RE}
0.0000	29.53210	29.53210	4.6606	13.94206	-0.03177	1.92932	-0.009346	1.2986

C_0 – Nugget effect; C_1 – Contribution; C_0+C_1 – sill; a – Range; a' – Practical range; ME – Mean error; SD_{ME} – Stand deviation of mean error; RE – Reduced mean error; SD_{RE} – Standard deviation of reduced mean errors

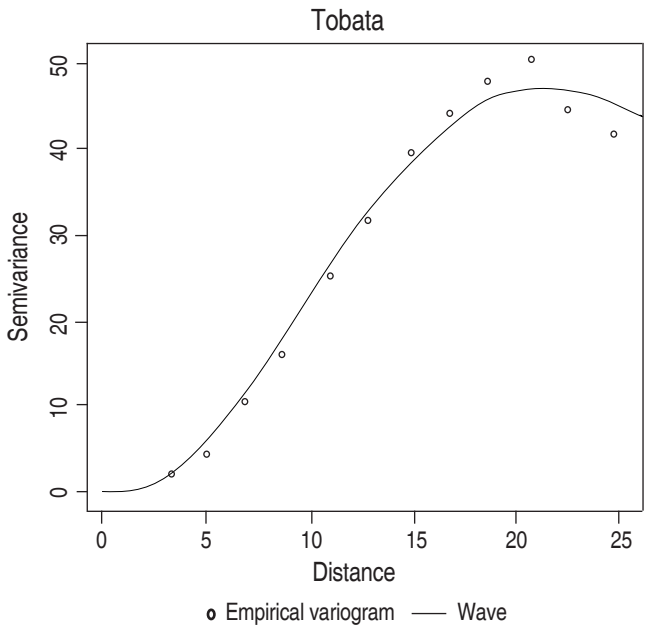

Figure 2. Semivariogram of the noise emitted by the micro-tractor under study.

(0,0), where the operator is positioned, the measured noise level was 91.20 dB (A), represented by the red color. This value can be considered high for daily exposure. The regulatory standard (NR 15) Ordinance 3,214/78 Ministry of Labor and Employment (MTbE) establishes that the maximum noise level allowed for 8 h of daily exposure is 85 dB(A). Above this limit, noise and disturbing human activities can cause serious damage to health (Silva *et al.,* 2004).

The approximate value of the maximum noise 91.0 dB (A), was observed at point (0, -2), which was measured near the micro-tractor (noise emitting source), represented by the red color in the map. According to ABNT (2020), the permitted limit was exceeded for a daily exposure of 8 h in all cases evaluated. In this way, it was noted that both

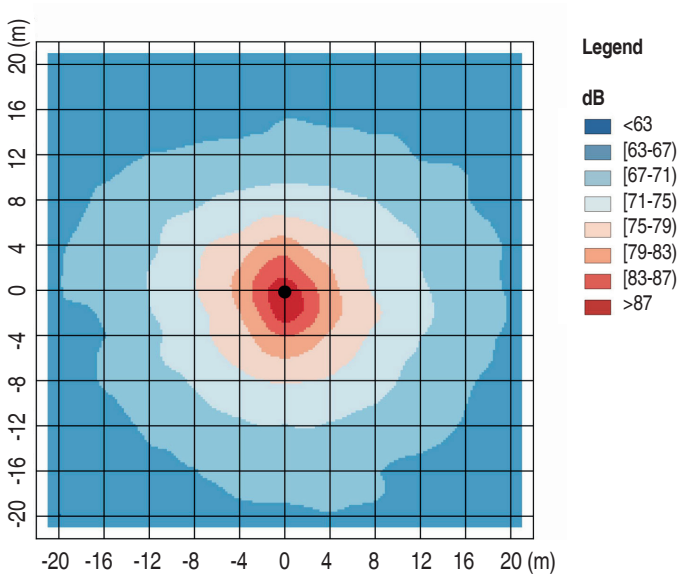

Figure 3. Spatial distribution map of the noise emitted by the studied micro-trator.

the operator of the evaluated tractor and the helper for the agricultural operation would be subjected to the harmful effects of the noise emitted by this machine.

It was found that operators need to wear hearing protection, that is, personal protective equipment (PPE). Therefore, the farther from the tractor, the more the color of the spots becomes bluish, which indicates the decrease in the noise level, thus becoming more appropriate for workers and not requiring the use of PPE. However, it is noted that even so, all workers involved in the operation of this machine use PPE. The minimum noise value produced by this tractor was about 59.0 dB (A) observed at point (-12, -20), that is, the furthest from the emitting source point.

Furthermore, observing the ABNT (2020) it is noted that the rural or similar employer must adopt ergonomic principles that aim at adapting working conditions to the psychophysiological characteristics of workers, to provide improvements in comfort conditions and safety at work, and also provides for the use of hearing protection, in this case, hearing protectors for activities with noise levels harmful to health.

Although the agricultural activity in Brazil is the most important since the colonial period until the middle of the 20th century, it had a significant advance for employees only from the Federal Constitution of 1988 in the seventh's article, where it guarantees rights to rural workers as well as urban workers and include unemployment insurance, the minimum wage, the severance pay for the length of service, the extra salary and the 8 h working day. Recently, ABNT (2020) presented progress for rural workers. It came to make the planning and development of agriculture, livestock, forestry, and aquaculture activities compatible with the safety and health of workers. With regard to ergonomics and safety in the use of agricultural machinery, this standard states that "all machines, equipment, implements, furniture, and tools must provide the worker with conditions of good posture, visualization, movement, and operation".

The majority of motocultivators use air-cooled single-cylinder engines. They are typically powered by diesel, with a four-stroke thermodynamic cycle and power ranging between 7,457 and 14,914 W. The total displacement varies between 250 and 500 (cm^3) with a maximum operating regime between 3000 and 3800 rpm (Márquez, 2012). In addition, when Gomes *et al.* (2013) evaluated the increase of motor rotation, they observed that this operation could cause an increase in the sound power level, but only at extreme speeds (2,200 rpm).

The values of the noise emitted by the studied machinery above 85 dB (A), for daily exposure of 8 h NR 15 (ABNT, 2021), are visualized in the operation regime, in general, up to 2 m on the right side and the front and 4 m at the rear and 2 m at the left side of the machine (Figure 4). The maximum noise emission value found in the tractor

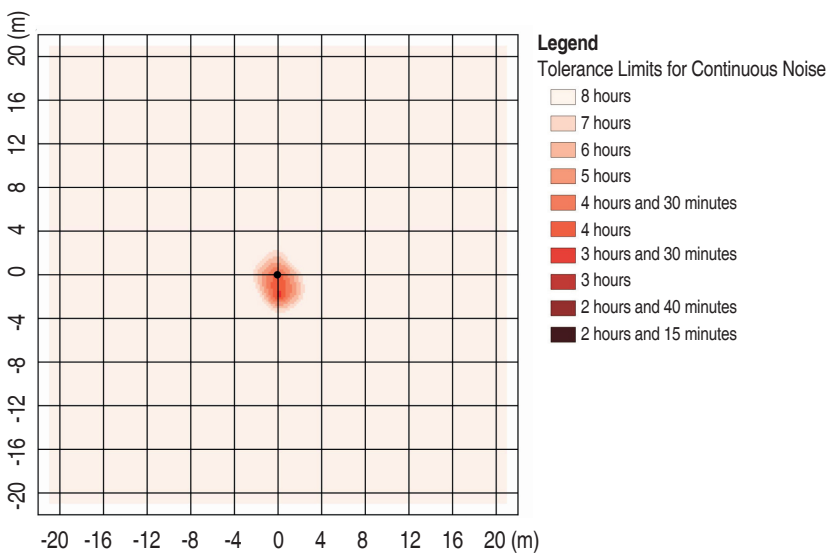

Figure 4. Map of the spatial distribution of the time of exposure to noise emitted by the tractor studied.

understudy was 91.20 dB(A), in the operator's seat (Figure 4). Thus, according to NR 15 (ABNT, 2021), the maximum exposure allowable daily is 3 h and 30 min. Thus, it is always recommended to use ear protectors when operating this tractor.

In Figure 5, the values related to noise levels obtained near the operator's ear are presented. At the front of the tractor, there was a similarity in the values of noise emitted by the tractor, indicating the noise emitted predominantly by the engine; however, only people located at distances greater than 4 m do not need to use PPE for shifts of 8 h. Figure 6 shows the control chart of the averages of noise

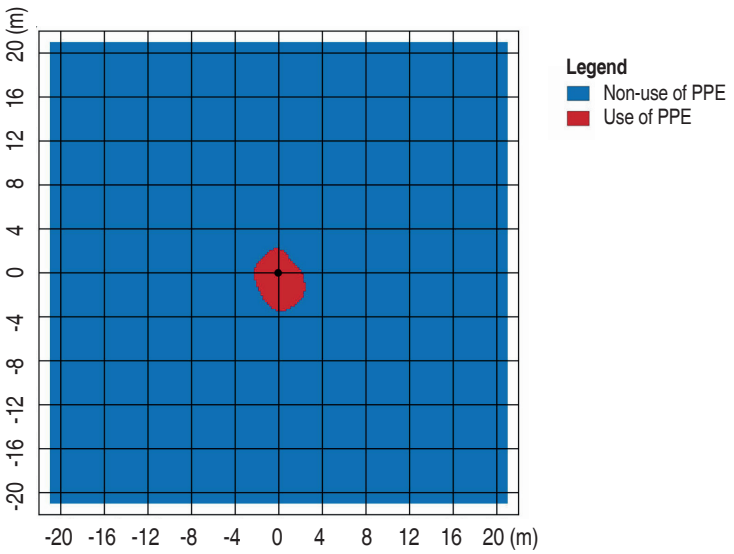

Figure 5. Map of necessity to use or not a Personal Protective Equipment (PPE).

level variable. It may be that the process is controlled, it means that the points at the upper and lower control limits (UCL and LCL) show that the process suffered variations from common causes. Furthermore, there are probably more points below the control limits for the micro-tractor studied, which show that the operation of this machinery was closer to the control. When the points outside these limits are observed, it is

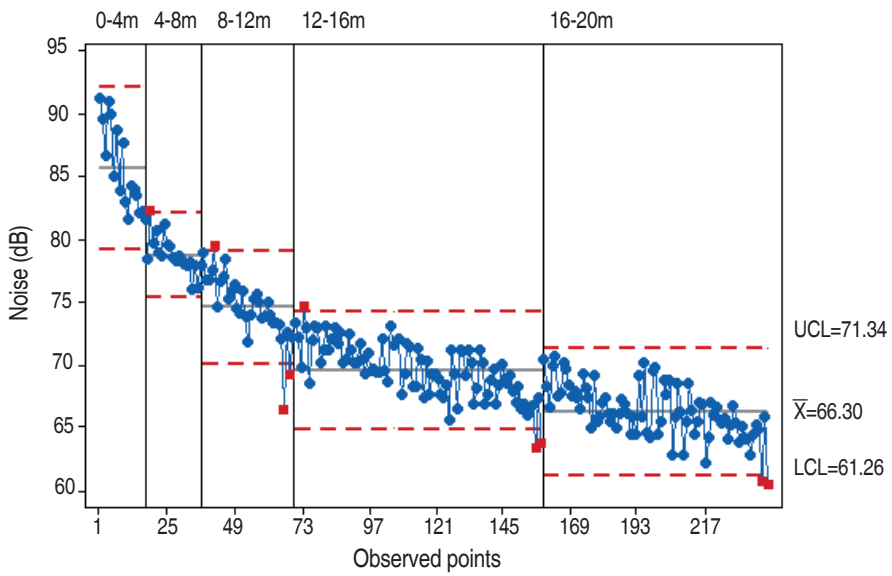

Figure 6. Control of noise levels of the studied tractor.

noted that these data were generated by incorrect data collection procedures or manufacturing years (Civardi, 2017). In contrast, when it comes to the sequences found, the main sequence pattern is when 7 (seven) or more consecutive points appear on just one side, below or above the midline (gray line). These sequences are more difficult to detect since it is necessary to analyze if the special causes will improve or harm the process (Nomelini *et al.,* 2009).

In Figure 6, this phenomenon did not occur in the analysis of the individual control chart of the machinery under study. It is considering that the periodicity is a detectable configuration in the long term. The points are distributed as a curve that has an alternating up and downtrend. This variation can be associated, for example, with interference variability.

An individual control chart was chosen to be used as an evaluation parameter along with geostatistical data. The individual control chart (Figure 6) shows that across all single value/observation relationships, evaluated across 441 collected points, some points are outside the control limit. It can be seen that through the points collected, the value of the highest noise peak in the experiment was 91.2 dB (A), as shown in Table 1.

Still, analyzing the control chart present in Figure 6, it is possible to observe the convergence of the distribution values around the average and note that most of these values are close to their respective stages during the process. The points distribution is explained according to the coefficients of asymmetry and kurtosis with positive values, in which the distribution of values tended to be more to the left and more flattened. Therefore, in a positively asymmetric distribution, the tendency is for positive deviations to be much more significant than the negative ones. Concerning the behavior of the tail being more flattened, it is linked to the kurtosis value of the distribution, which is greater than 0.263 (Table 1), the distribution is flatter than the normal curve of the same area, and it is said to be a platicurtic curve. (Sindelar *et al.,* 2014).

The individual control chart confirms that there were outliers in this case, as there were peaks in values within the subgroup and it was affecting the sample amplitude. Statistically, outliers are considered data outside the standard of the studied population; in this case, these data are considered interference peaks, which occur due to the influence of some external factor (Silva, 2019).

To sum up, Figures 3, 4, 5, and 6 illustrate the spatial distribution of noise that this machine may generate. These maps, based on the values recommended by NR 15 (ABNT, 2021) have fundamental importance for the management of acoustic health during the use of the agricultural machine, as they facilitate the understanding of the risks caused by noise exposure.

CONCLUSIONS

It was possible to characterize the structure and magnitude of spatial dependence of noise level emitted by the micro-tractor using the Wave model and its spatial distribution map through kriging.

The process has been studied in detail by the process control charts (SPC). It allowed identifying special causes according to variations in the process. Thus, it was possible to develop maps of the noise level emitted by the studied micro-tractor such as Spatial distribution map, Map of the spatial distribution of the time of noise exposure, and Map of necessity to use or not a Personal Protective Equipment (PPE), contributing to the management of acoustic health during the operation of the studied tractor.

For further studies, it is recommended to use a decibel meter and a dosimeter to improve the analyses. Also, it is useful to do this kind of study in other agricultural tractors, machines, and implements.

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Physiological adaptation indicators of three Colombian Creole cattle breeds

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Indicadores fisiológicos de adaptación en tres razas de bovinos criollos colombianos

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ABSTRACT

The present study aimed to determine the physiological response and its relationship with the caloric impact (stress) index of three breeds of Colombian Creole cattle (Hartón del Valle, Blanco Orejinegro y Sanmartinero). These breeds have been considered adapted to low-tropical conditions and important to the sustainable meat and milk production in Colombia. To determine two heat tolerance indexes and two hormones and their relationship to the physiological response to heat stress in three Colombian Creole cattle breeds, Pearson's correlation methodology and mean comparison study were used. The adaptability indexes such as temperature-humidity index (ITH) and heat load index (HLI) and cortisol and T3 hormones were estimated and subsequently, the indexes were correlated with the hormones using the Pearson methodology, also the mean comparison study was used. Physiological variables were analyzed such as heart rate, respiratory rate, rumen patterns, and rectal temperature; during the study, serum concentrations of cortisol and triiodothyronine hormones were detected in seven different instances during the rainy season in the Piedemonte Llanero. ITH and HLI were used as indicators of thermal compensation. The HLI appeared to be a better indicator when the environmental conditions included wind speed and solar radiation. No variations were found between the breeds ($P \geq 0.05$), while the measurement periods did show variations ($P \leq 0.05$). It may conclude that the HLI provides better information to study physiological parameters and it may confirm that the animals are considered adapted to the conditions of the local environment.

Keywords:
Climate change
Colombian creole cattle
Heat Load Index
Stress
Temperature-Humidity Index

RESUMEN

Se determinó la respuesta fisiológica y su relación con el índice de impacto (estrés) calórico en tres razas de bovinos criollos colombianos (Hartón del Valle, Blanco Orejinegro y Sanmartinero). Dichas razas son consideradas adaptadas a las condiciones de trópico bajo e importantes para la producción de carne y leche en Colombia. El objetivo del estudio fue determinar dos índices de tolerancia al calor y dos hormonas y su relación con la respuesta fisiológica al estrés calórico en tres razas criollas colombianas usando análisis de correlación y comparación de medias. Los índices de adaptabilidad ITH y HLI, las hormonas cortisol y T3 se estimaron y estos se correlacionaron con las hormonas y se compararon las medias. Se analizaron las variables fisiológicas: frecuencia cardíaca, respiratoria y ruminal y, temperatura rectal; igualmente se determinaron las concentraciones séricas de las hormonas cortisol y triyodotironina, en siete momentos en la época de lluvias en el Piedemonte Llanero. El índice de temperatura y humedad (ITH) y el índice de carga calórica (HLI) fueron empleados como indicadores de compensación térmica. El HLI mostró mejor relación con las condiciones ambientales al incluir el índice la velocidad del viento y la radiación solar. No se encontraron variaciones entre los grupos raciales ($P \geq 0,05$), mientras que los periodos de medición sí presentaron variaciones ($P \leq 0,05$). Se concluye que el HLI proporciona mejor información para el estudio de los parámetros fisiológicos y éstos reflejan la adaptación de los animales a las condiciones del medio.

Palabras clave:
Cambio climático
Bovinos criollos colombianos
Índice de carga calórica
Estrés
Índice de temperatura y humedad

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Euroamerican genetic resources are an important foundation of biodiversity and their differentiation patterns must be studied and conserved (Sponenberg, 2018). In Colombia, eight racial groups derived from animals that arrived on the second and third voyages of Columbus in the 16th century have been recognized, these constitute a valuable genetic bank of the *Bos taurus*, adapted to the Colombian tropical lowlands (Martínez *et al.,* 2009). Physiological adaptation of these bovines is reflected in their greater longevity and better reproductive rates achieved in difficult ecological environments, with high temperature and humidity, the presence of external and internal parasites, and the low nutritional quality of grazing forages. On the other hand, climate change opens a special window to analyze anthropogenic livestock farming processes (Saiz, 2010). However, in our environment, the adaptation processes of the Colombian Creole breeds and their potential against global warming have not been studied, since the adaptive process responds to homeostatic mechanisms, developed during more than five centuries of interaction with the tropical environment (Núñez-Domínguez *et al.,* 2016).

In cattle, the thermal stabilization mechanisms against a warm environment include the control of excess heat and respiratory rate, regulation of blood flow to the skin, and regulation of the consumption of dry matter and water (Sejian *et al*., 2018); variables that have been quantified according to their genetic component, through the determination of hormones, metabolites, or specific physiological parameters (Franzoni *et al*., 2018). Cortisol and thyroid hormones are indicators associated with chronic stress or thermal regulation (Kamal *et al*., 2018; Campos *et al.,* 2004). Since the temperature of an animal depends on the calories produced per unit mass and the rate of heat exchange, which in turn depends on the production of metabolic heat, the heat gains from external sources, and the rate of heat loss to the environment (Kamal *et al*., 2018), in the case of warm environments, body temperature tends to increase above normal values because of the effect ambient temperatures and solar radiation, as well as the heat from the ground. Air temperature and solar charge determine the speed with which an organism exchanges heat with the environment (Da Silva *et al*., 2015).

When the ambient temperature rises, bovines increase their respiratory rate (RF), pulmonary ventilation, and respiratory vaporization, but when temperatures reach 26 °C for *Bos taurus* and 35 °C for *Bos indicus*, especially when the relative humidity is greater than 75%, these mechanisms are not capable of dissipating all excess heat, given that the higher the relative humidity, the lower the ability to lose heat by convection (Sejian *et al*., 2018); for this reason, determining the caloric load indexes allows the understanding of the relationships derived from the physiological responses (ruminal, cardiac and respiratory frequencies, rectal temperature, bodily heat exchange) to climatic challenges such as wind speed, sunlight, humidity and air temperature (Polsky and von Keyserlingk, 2017), to elucidate the physiological mechanisms of adaptation of local native cattle. Consequently, this study aimed to determine two heat tolerance indexes and two hormones and their relationship to the physiological response to heat stress in three Colombian Creole cattle breeds using Pearson's correlation methodology and mean comparison study.

MATERIALS AND METHODS

The experiment was carried out at the La Libertad research center of AGROSAVIA in Villavicencio located at 4°03´ north latitude and 73°46´ west longitude, at 330 masl, the annual average temperature is 26 °C, relative humidity of 80%, the annual precipitation in this geographic area in the last 28 years exceeds 2900 mm, with a rainy season between April and November, followed by a dry period between December and March (IDEAM, 2017).

Blood samples were collected for hormones analysis, and physiological parameters were determined in four Sanmartinero breed cows, six Blanco Orejinegro breed cows, and five Hartón del Valle breed cows (Figure 1 A-C); with an age range of 3-12 years, and between one and five calvings, grazing in *Brachiaria decumbens* pastures with access to mineralized salt and water *ad libitum*.

Table 1 presents the weight, body condition score, production, and composition of the milk of the experimental cows. These animals were subjected to health prevention and control programs following the regular schedules and plans provided by the center, which included vaccination against foot-and-mouth disease and brucellosis in females and control of external and internal parasites twice a year for all

Figure 1. Colombian creole cattle breeds. A. Hartón del Valle. B. Sanmartinero. C. Blanco orejinegro. (Photographaps by G. Onofre R.)

animals. All experimental procedures were reviewed and approved by the animal ethics committee at the Faculty of Agricultural Sciences of La Salle University.

The collection of samples was carried out between April and September (rainy season) with seven evaluation periods, with an interval of 15 to 30 days. After a 60 min break, females entered the holding area, located under an artificial shade where cows could recognize the environment and relax for 15 min before starting the measurement of the physiological parameters and collecting blood samples carried out between 11:30 and 13:00 hours. Each cow's heart rate was measured by auscultation for 1 min, respiratory rate by observation of the lateral flank for 1 min also, ruminal motility by observing the left flank fossa for 2 min, and rectal temperature with a mercury thermometer for 3 min. Blood samples were taken by puncture of the coccygeal vein using 22-gauge needles, 25 mm long, and vacuum plastic tubes (Vacutainer®). Blood samples were centrifuged within 1 h after sampling to separate serum and were stored at -80 °C until processing in the laboratory. Cortisol and T3 hormones were analyzed by solid-phase radioimmunoassay (RIA), with a commercial kit (Siemens) labeled with ^{125}I radioactive isotope and cortisol by RIA through DPC kits (Los Angeles, CA); Reading was performed with a Nucleonics single-well equipment (Berkeley, CA) and the values of each hormone were obtained using the RIACALC software, from the University of Guelph (Canada).

Table 1. Mean values of body weight, body condition, dairy milk production, and lactation and milk composition of experimental cows

Variable	Hartón del Valle	Blanco Orejinegro	Sanmartinero
Body weight (kg)	475	520	450
Body condition score (1 to 5 scale)	3.0	3.0	3.0
Dairy milk production (kg d^{-1})	3.9	3.1	2.4
Total production per lactation (kg)	1002	737	633
Milk fat (g 100 g^{-1})	3.6	4.2	3.1
Milk protein (g 100 g^{-1})	3.5	3.6	3.7
Total solids (g 100 g^{-1})	12.6	13.5	12.8

The climatological data of the observation period were obtained from the meteorological station N°70 "La Libertad" of IDEAM located in the Research Center at latitude 4°04'N and longitude 73°28W. The information evaluated included the records of environmental temperature in the dry bulb, black bulb, relative humidity, wind speed, and solar radiation. With the data obtained, three ranges of caloric load (HLI) were established by Lees *et al.* (2019): 1 (low), 60-65; 2 (medium), 65.1-70, and 3 (high), 71-75.

The temperature-humidity index (ITH) was determined according to the equation: ITH = (1.8Ta + 32) – (0.55 – 0.0055RH) x (1.8Ta–26.8) HLIBG>25 = 8.62 + (0.38RH) + (1.55BG) – (0.5WS) + [$e^{2.4}$–WS]; where Ta is the ambient temperature (°C) and RH is the relative humidity expressed as a percentage (Dikmen and Hansen, 2009). To include the variables black bulb temperature and wind speed, the index Heat Load Index (HLI) was used. The HLI has two measurement points, for this experiment the (HLIBG) corresponding to the ambient temperature measured in a dry-bulb greater than 25 °C was used, which is calculated with the formula: HLIBG>25 = 8.62 + (0.38RH) + (1.55BG) – (0.5WS) + [$e^{2.4}$–WS]; where RH is the relative humidity, BG is black bulb thermometer temperature, "e" is the base of the natural logarithm (e=2.71828) (Das *et al.,* 2016) and WS is wind speed.

The experimental design and statistical analysis included a generalized linear model (GLM) and analysis of variance with three treatments (breeds) and seven periods, analyzing the caloric load factor. For all variables, measures of central tendency, variance, and standard deviation were calculated as measures of dispersion. The existence of possible associations between the variables studied was analyzed using Pearson's moment correlation coefficient. The statistical program SPSS version 23.0 (SPSS, 2015) was used for the analysis. Due to the homogeneity of the environment and having a different number of experimental animals for each treatment, the data was organized in a completely random arrangement (Petrie and Watson, 2013). The test was assumed to be significant if the probability associated with it was less than or equal to 5%. Duncan's test was used as a means comparison test.

In order to know the behavior of the physiological variables regarding the indexes of climatic adaptation (HLI and ITH), in the first phase, Spearman correlations were estimated to evaluate the relationships between the variables (P<0.05). Subsequently, multiple linear regression was performed, using the mixed model methodology. The regressors were all physiological variables (cortisol, T3, breed, rectal temperature, ruminal, heart, and respiratory frequency), the adaptation index (HLI or ITH) was the response variable, while the cow was considered as a random effect. In the first modeling attempts, it was observed in the partial residuals that the variable "ruminal frequency" included a quadratic behavior, so it was estimated and included as a regressor. To identify the physiological variables that most influenced the indexes, the "Backward" variable elimination method was used at P<0.05.

RESULTS AND DISCUSSION

Table 2 describes the climatic conditions that occurred during the experimental period, on the sampling days in which the physiological parameters were determined in the experimental animals. The ITH was higher ($P \leq 0.05$) in period 7 and lower in period 6 (Table 2), coinciding with the highest records of maximum temperature and relative humidity of 75%, records compatible with severed stress; however, the physiological parameters were found within the ranges expected for creole cows in conditions of the tropical lowlands (Table 3), but with

Table 2. Climatic variables, ITH and HLI associated with seven instances between April and September in the Experimental Center La Libertad (Villavicencio- Colombia).

*	1	2	3	4	5	6	7
Maximum Temperature (° C)	32.0 c	31.0 bc	31.6 bc	32.4 c	29.6 b	26.0 d	33.4 a
Minimum Temperature (° C)	22.2 b	22.0 b	21.6 c	23.4 a	22.2 b	22.0 b	23.2 a
Relative Humidity (%)	82 b	86 b	87.3 a	69.5 d	67.5 d	89 a	75 c
Win speed (m seg^{-1})	6.12 c	6.75 bc	7.28 bc	7.86 bc	11.07 a	2.57 e	4.68 d
Solar brightness (h)	6.6 b	9.4 a	4.1 c	6.5 b	3.7 c	1.1 e	2.6 d
HLI[1]	57.92 c	66.34 a	61.26 b	62.09 b	60.41 b	58.11 c	59.48 b
ITH[2]	78.5 b	78.9 b	78.3 b	78.2 b	75.9 c	74.2 c	81.2 a

*Sampling dates: 1=08/04; 2=22/04; 3=06/05; 4=20/05; 5=08/07; 6=12/08; 7=30/09

Different letters on each line indicate a significant difference ($P \leq 0.05$), through the Duncan test.

[1] HLI= Heat Load Index (Caloric Load Index). [2] ITH= Humidity Temperature Index

changes ($P \leq 0.05$) throughout the evaluation periods. The mean cortisol concentration found was 151.7 nmol L^{-1} and the greatest concentration (233.8 nmol L^{-1}) was found in period 3 and was greater ($P \leq 0.05$) than in periods 1, 6, and 7. The average value of the T3 concentration was 1.46 nmol L^{-1} but there were no differences between periods except for periods 3 with the greatest concentration (1.92 nmol L^{-1}) and 5 with the lowest (0.94 nmol L^{-1}) ($P \geq 0.05$). The HLI value was greater ($P \leq 0.05$) in period 2 and lower in period one, but there was evidence that the climatic variables recorded in the sampling periods corresponded to an HLI, from group 1 or lower in the range proposed (between 60 to 65).

Pearson's correlation coefficients between HLI and ITH were significant ($P<0.001$) with a value of 0.55. Since it is a medium and positive correlation, the HLI index was adopted because it included a greater number of climatic variables, which would theoretically make it more accurate.

The results for multiple linear regression showed that 3 variables T3 ($P<0.0502$), heart rate ($P<0.0102$), and quadratic ruminal frequency ($P<0.0167$) were equally significant for both indexes, although with a low R^2 (0.21). The only difference between the estimated models was found in the intercept 95.38 and 78.38 for ITH and HLI, respectively).

$$HLI = 78.38 \times -2.53 \times T3 - 0.13 \times \text{heart rate} - 0.54 \times \text{ruminal frequency}^2$$

$$ITH = 95.38 \times -2.53 \times T3 - 0.13 \times \text{heart rate} - 0.54 \times \text{ruminal frequency}^2$$

Table 3. Serum concentrations of cortisol, T3, physiological constants, and HLI, on seven different occasions between April and September (rainy season) in Colombian Creole cattle in the Piedemonte Llanero.

	1	2	3	4	5	6	7
Cortisol (nmol L^{-1})	126.6 b	159.5 ab	233.8 a	208.5 ab	162.0 ab	119.9 c	126.4 bc
T3 (nmol L^{-1})	1.46 ab	1.44 ab	1.92 a	1.45 ab	0.94 b	1.51 a	1.51 a
Heart rate (b min^{-1})	72.67 b	74.00 b	72.09 b	56.11 d	87.25 a	43.12 e	63.73 c
Respiratory rate (Mov min^{-1})	65.7 a	61.1 ab	51.0 de	42.2 de	29.9 f	33.1 e	52.4 cd
Ruminal movements (Mov min^{-1})	2.7 cd	2.8 cd	2.7 cd	2.7 cd	1.9 e	2.4 de	3.0 a
Rectal Temperature (°C)	39.75 a	39.81 a	39.54 a	39.50 a	39.35 a	39.41 a	39.66 a
HLI[1]	57.92 c	66.34 a	61.26 b	62.09 b	60.41 b	58.11 c	59.48 b

Different letters on each line indicate a significant difference ($P \leq 0.05$) through Duncan's Test.
[1] HLI= Heat Load Index (Caloric Load Index)

The variables analyzed between hormones, climatic information, and the HLI did not correlate ($P \geq 0.05$) between physiological parameters and the caloric load index (HLI).

Blood cortisol concentration was greater than those reported by Campos *et al.* (2009), in a study of metabolic behavior in the peripartum of Hartón del Valle cows, under tropical conditions, and exceed those found in the same breed, but with lactating cows, whose average values fluctuated between 94 - 94.1 nmol L^{-1} (Campos *et al.,* 2012). It showed a different racial behavior, similar to the case of the Sanmartinero racial group. Other values can be found in cattle; however, these are not completely comparable due to the racial type and the climatic conditions in which they are determined (Carvalho and Dupuy, 2017).

The blood concentration of T3 in the cows of this study was consistent with those found in the Colombian Creole lactating cows described by Campos *et al.* (2004). The role of thyroid hormones in the face of stress has been discussed (Kamal *et al.,* 2018), finding that lactating cows subjected to acute heat stress report values of 0.95 nmol L^{-1}. The thyroid and endocrine metabolic responses to climate change are not immediate (Gaughan *et al.,* 2019) and therefore, despite the existence of significant differences between sampling periods in the present study, there

is no evidence indicating a direct relationship between caloric load and thyroid response.

First of all, there was no relationship between physiological parameters (respiratory, heart, and ruminal rates, and temperature) and blood cortisol concentrations within normal concentrations; the absence of a relationship may be because the determinations of the physiological constants were punctual (seven specific moments), when a process of adaptation should generally respond to a wider measurement window, which is not always possible because of the costs and permanent handling of animals that affect cortisol concentrations.

Secondly, the time of the year in which sampling was carried out (April to September -Table 2), corresponds to the rainy or wet season, which in the ecozone of study, is of monomodal type, where the rainy and dry seasons are markedly contrasting (Amézquita *et al.,* 2013), which could generate lower physiological efforts in the animals by having less radiation and better compensation for heat loss by refrigeration.

Thirdly, the number of experimental animals is an important limitation to extract information that can be extrapolated as a general parameter for Colombian Creole cattle, since only three of the eight breeds recognized as Colombian were studied in the experiment (Martínez *et al*., 2009), and among these groups, there was a limited and heterogeneous number of individuals. These cows came from the germplasm banks of the Research Center which limited access to a greater number of animals per breed, which have a reduced inventory; however, the responses for breeds were consistent throughout the evaluation period, which supports the results obtained.

The HLI was not different ($P \geq 0.05$) between dates in which the physiological parameters were determined. This index was considered low (level 1), with no environmental impact, and corresponds to the non-extreme climatic conditions registered, which were verified with the traditional ITH, that shows that the work area (Piedemonte Llanero) at the time of the measurements presented an ITH corresponding to the limit between the zone of moderate stress and severe stress zone, which is not considered significant for animals adapted to these environmental conditions. Only the seventh sample presented a qualification of the ITH as severe stress because in this period the environmental temperature was the greatest observed (33.4 °C) and the relative humidity was 75%, which generates a high-stress value according to the value of ITH for *Bos taurus* cattle under these conditions (Polsky and von Keyserlingk, 2017); however, the physiological responses of the cows were not altered, which raises questions about the value of ITH as an indicator of physiological response in bovines in the tropics and subtropics, as has been proposed by Dikmen and Hansen (2009).

According to Lees *et al.* (2018), the development of a heat stress index for livestock must be based on biological factors, which generates the need for a high number of data to develop and test an index. The biological parameter used should also be easy to measure and be a good indicator of the heat load. The physiological and behavioral changes were reliable indicators of the state of the heat load, demonstrated in the present work that the experimental animals were in conditions of adaptation since they did not present behavioral alterations or changes in their physiological parameters.

Heart frequency was between 40 and 94 beats per min. There was a positive correlation between heart frequency and heat load. Other authors found the same tendency (Peña *et al.,* 2011), in Patagonian Creole cattle. On the other hand, various alterations have been found in the physiological patterns that originated in adaptive changes in cattle under conditions of humidity and temperature similar to those of the experiment (Parra-Herrera *et al.,* 2017).

The rectal temperature remained within normal limits, which is to be expected if the thermoregulatory mechanisms are functioning. There were no differences in rectal temperature between treatments, although it increased at the maximum value with a greater caloric load. In the same sense, efficiency in thermal regulation is known when critical stress situations are not faced (Sejian *et al.,* 2018).

Regarding ruminal motility, the comparison presented differences ($P \leq 0.05$) in the number of ruminal movements under different caloric loads and, the linear

regression analysis described a slight tendency to increase with greater caloric load, which would indicate that the consumption of dry matter did not affect, since the ingestion of grass was not compromised, which for native breeds of *Bos taurus* origin is positive because indirectly it can be associated with adaptation.

When the mechanisms of thermoregulatory response act in the bovine, ruminal movements are not altered, and this can clarify the significant positive correlation between respiratory frequency and skin heat loss processes. Pulmonary ventilation showed a trend line indicating that the higher the caloric index, the higher the respiratory frequency (significant correlation of 0.556). The reason why the high respiratory rate presented at high temperatures is that the cattle use pulmonary ventilation as a heat dissipation mechanism along with transpiration and behavior changes seeking a lower exposure to solar radiation (Das *et al*., 2016).

The physiological parameters (Table 3) presented values within the range known as "normal" for animals under the conditions of the experiment and did not present extreme outliers, except for the fifth sampling focal point, where there is no expected relationship between respiratory and heart frequency. The values for all animals did not present differences ($P \geq 0.05$) between the racial groups studied. This could indicate that under the experimental conditions the animals of the three racial groups exhibited similar physiological patterns and therefore high similarity of adaptive behavior associated with the interaction with the environment where they were kept. The main limitations for a conclusive connection between the relationship for adaptation with physiological variables lie in the number of animals included in the study and the homogeneity of animals per group.

Similar results were observed in Brazil by Lima *et al*. (2020) when evaluated the effect of temperature on Caracú (creole) and Nelore males in three different periods: morning (8:00 to 10:00), afternoon in the sun, and afternoon in a shaded environment (11:00 to 13:00). The animals of the two breeds exhibited no significant changes in heart rate, respiration rate, and cortisol level for the maintenance of thermal homeostasis. Researchers concluded that Caracú and Nellore are considered tolerant to sun exposure, indicating adaptation of these breeds to the high-temperature environments of the tropics. In northern Ethiopia was evaluated the effect of the season (dry and rainy season) on the physiological response to heat stress in the creole Fogera cattle (Abera *et al*., 2021). During the dry season was observed an increase in respiratory and heart rate, but not in rectal temperature.

Some authors suggest that is necessary a period of adaptation to evaluate response to heat stress. In India Yadav *et al*. (2021) developed a study to assess the acclimatization dynamics in dry crossbred creole cows (Haryana × Brown Swiss, Holstein, and Jersey). Cows were exposed daily to 25 °C and 40°C with a relative humidity of 40–50% in a climatic chamber from 10:00 to 15:00 h, for 21 days. Rectal temperature, respiratory and heart rate increased at 40 °C compared to 25 °C, but there was no effect on the T3 hormone. Acclimatization response in respiratory rate was first observed on day 11 and for T4 and cortisol hormones levels on day 11 and 16, respectively. The authors concluded that in extreme heat stress in creole crossbred cows is necessary a period of adaptation of 6 to 21 days for acclimatization to heat stress.

However, some physiological mechanisms of response to heat stress in tropical conditions are different between *Bos taurus* and *Bos indicus* or crossbred cattle (*Bos indicus* × *Bos taurus*). Moura *et al*. (2021) compared the physiological responses of an F1, Nellore x Angus to Nellore cattle kept under moderate thermal conditions (air temperature 16 to 30 °C). Slight differences in thermal storage and daily fluctuation of daytime rectal temperature (07:30 and 16:30), were observed in Nellore (39 to 39.30 °C) compared to Nellore x Angus (39.12 to 39.62 °C). In addition, a study in Brazil on Nellore heifers found that heat stress (34 °C) increased heart and respiratory rates (Moreno *et al*., 2021).

The climatic variables (Table 2) reflect the corresponding rainy season; thus, the relative humidity presented high values, and in at least one day a low wind speed was associated with greater rainfall, these values have been described by various authors for the geographical area called "Piedemonte Llanero" comprised approximately between 3°65' and 4°15' on the eastern slope of the eastern mountain range of Colombia (Amézquita *et al.,* 2013).

The relationship of climatic variables with physiological behavior was analyzed through the correlation between ITH and HLI, to integrate the animal response to the climatic conditions in which they live. The implications of unfavorable environmental conditions for livestock are presented in studies that describe how environmental stress negatively affects animal health and productivity (Herbut *et al.,* 2018; Polsky and von Keyserlingk 2017).

The first reference to the behavior of Creole cattle in various climatic conditions was based only on temperature and humidity changes and mechanisms morphological, behaviors, neuroendocrine, and cellular responses (Angel *et al*., 2018), which, although not invalidated, leaves reasonable doubt as to its current predictive value on physiological behavior. This paper uses the HLI that also contemplates solar radiation and wind speed, as a dynamic way to look for the best indicators that allow analyzing the physiological processes of adaptation (Das *et al.,* 2016).

The concept of the caloric load index generates a better understanding of the adaptation processes since the inclusion of the two climatic variables (black bulb temperature, which measures radiant energy or solar radiation, and wind speed), allows us to analyze the rates of heat loss or gain by the animal in its environment since this impact modifies the indexes in which the animal response is measured (Herbut *et al.,* 2018). Likewise, the low HLI values allow us to recognize that there are dynamic processes of physiological compensation in a moderately adverse environment (low HLI); on the contrary, the recording through ITH between 74 and 81.2 (Table 2) places the animals in front of dangerous classifications of alert, because they are close to the known limits for severe stress, which does not agree with the determined physiological constants.

When the simple interactions between biological variables were studied, through Pearson's correlation (Table 4), there is a wide variation between the values and statistical significance found, which does not always present a close correlation with the expected homeostatic behaviors or with the factor's compensatory physiological measurements between one measurement and another. Even though

Table 4. Pearson's correlations between physiological parameters, hormonal concentrations, and HLI in Colombian Creole bovines in Piedemonte (lowlands).

	HLI[1]	Cortisol	T[3]	HR[2]	ReR[3]	RuM[4]	RT[5]
HLI	1						
Cortisol	0.088	1					
T3	0.034	0.066	1				
Heart rate	0.361*	0.241*	-0.064	1			
Respiratory rate	0.556*	0.095	0.16	0.099	1		
Ruminal rate	0.064*	0.121	0.206	0.244*	-0.181	1	
Rectal temperature	0.161*	0.088	-0.054	0.057	0.209	0.27	1

* Values with an asterisk presented statistical significance $P \leq 0.05$)
[1] Heat Load Index; [2] Heart rate; [3] Respiratory rate; [4] Ruminal movements; [5] Rectal temperature

the present study found a better relationship between the HLI and physiological parameters compared to ITH, the applicability of the ITH had been demonstrated when the index was related to production traits. A study in Tanzania evaluated the effect of heat stress on milk production using the ITH on test-day milk records of smallholder dairy cattle herds (Ekine-Dzivenu *et al*., 2020), cows experienced heat stress between ITH values of 67 and 76. Similar results were observed in dairy cows in the tropical conditions of Costa Rica with a decrease in milk production when values of ITH exceed 72 (Ruiz-Jaramillo *et al*., 2019). In Italy Maggiolino *et al*. (2020) determined if a threshold of the ITH exists for milk production traits in Italian Brown Swiss dairy cows, researchers found that the Brown Swiss breed had higher thermal tolerance compared to Holstein cows. As ITH rose, Brown Swiss cows tended to produce the same volume of milk, but with a decreasing quality concerning components. This particular situation could be

similar in creole cows adapted to tropical conditions for Fogera cattle of Ethiopia. Abera *et al*. (2021) found that the ITH value of 66 was considered optimum for high weight gain and normal physiological response to heat stress. The literature reports several thermal indexes in addition to the HLI that can be used to predict the degree of heat stress on cattle by relating environmental variables to physiological parameters. Researchers in Brazil developed a model based on an artificial neural network, for individual assessment of the level of thermal stress in feedlot finishing Nellore cattle which includes both weather (dry and wet bulb temperature) and animal variables (rectal temperature, respiratory rate, and infrared body surface temperature). The thermal stress predicted by the neural model was correlated with rectal temperature, and the prediction was higher than the ITH method (Vieira de Sousa *et al*., 2018). In addition, the Equivalent Temperature Index for Cattle (ETIC), which includes air temperature, relative humidity, air velocity, and solar radiation and their interactions was developed in 2018 for dairy cattle in Europe. The ETIC predicted better skin temperatures, core-body temperatures, and respiration rates compared to the ITH, the heat load index, and the comprehensive climate index (Wang *et al*., 2018).

CONCLUSIONS

The physiological and endocrine relationships in face of climatic variations were reflected in the HLI, describing that the latter presents a correlation with the physiological parameters, although its value was low (0.03–0.55; $P \leq 0.05$). The assessment of associations when cortisol was determined and closely related to the time and degree of the stress conditioner that is present and to which the animals respond (0.06–0.24; $P \leq 0.05$). The multiple linear regression showed that 3 variables T3 ($P<0.0502$), heart rate ($P<0.0102$), and quadratic ruminal frequency ($P<0.0167$) were equally significant for HLI and ITH indexes. In the case of the present experiment, no acute and severe challenge could have generated an elevation in the concentration of cortisol (126.6–233.8 nmol L^{-1}), nor extreme changes in the values registered of the physiological parameters (Heart rate, 43.1-87.7 beats min^{-1}; respiratory rate, 29.9–65.7, mov min^{-1}; ruminal movements, 1.9–3.0 min^{-1}; and rectal temperature, 39.3–39.8 °C). Studies in physiology such as the present one, are essential to know the biological mechanisms of adaptation of Colombian Creole cattle.

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Use of Ultrafiltration Technology to Concentrate Whey Proteins after White Cheese Manufacturing

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Uso de la tecnología de ultrafiltración para concentrar proteínas de suero después de la fabricación de queso blanco

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ABSTRACT

Keywords:
Cheese manufacturing
Protein concentrate
Ultrafiltration membrane technology
Whey

Dairy industry generates contamination due to whey dumping from the manufacture of coagulated products. Ultrafiltration technology has been extensively studied in acid whey; however, research on sweet whey —which is obtained from the production of fresh white cheese (Campesino cheese)— is scarce. The objective of this study was to concentrate sweet whey proteins by ultrafiltration and to evaluate the process conditions. A polyethersulfone membrane with a molecular weight cut-off of 10 kDa was used. The effect of volumetric concentration factor between 5 and 18, transmembrane pressure between 2.5 and 5 bar was evaluated on the permeate flow, protein retention coefficient, and retention yield using a response surface methodology. The process optimization was carried out in that same range. Protein and fat were concentrated and underwent ultrafiltration; however, a less stable system was obtained. A higher concentration of protein can result in more collisions between molecules, thus generating flocculation. Whey protein concentrates had 18.2% of total solids out of which protein represents 45%.

RESUMEN

Palabras clave:
Fabricación de queso
Concentrado de proteínas
Tecnología de membranas de ultrafiltración
Lactosuero

La industria láctea genera contaminación por el vertimiento de lactosueros resultantes de la elaboración de productos coagulados. La tecnología de ultrafiltración se ha estudiado ampliamente en suero ácido; sin embargo, la investigación es escasa en suero dulce, el cual se obtiene de la producción de queso blanco fresco (queso campesino). El objetivo de este estudio fue concentrar por ultrafiltración las proteínas de lactosuero dulce evaluando condiciones de proceso. Se usó una membrana de polietersulfona con tamaño molecular de corte de 10 kDa. El efecto del factor concentración volumétrico entre 5 y 18, y la presión transmembrana entre 2,5 y 5 bar fueron evaluados sobre el flujo de permeado, coeficiente de retención y rendimiento de retención de la proteína en una metodología de superficie de respuesta. En ese mismo rango se realizó la optimización del proceso. Las proteínas y grasas se concentraron aplicando ultrafiltración; sin embargo, se obtuvo un sistema menos estable ya que una mayor concentración de proteína puede resultar en más colisiones entre moléculas, generando floculación. El concentrado de proteína de suero tuvo 18,2% de sólidos totales, de los cuales la proteína representa el 45%.

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Dairy industry produces whey as a result of the enzymatic or acid coagulation of milk proteins. This co-product generates environmental contamination problems, corroborated by indicators such as biochemical oxygen demand (BOD) that varies between 30000 and 50000 mg kg^{-1}, which is mainly due to its lactose and whey protein content. This dumping without previous treatments generates phenomena such as oxygen consumption, waterproofing, eutrophication, toxicity, among others, in bodies of water (Prazeres *et al.,* 2012; Giroux *et al.,* 2015).

Whey is obtained after removing casein from milk. Casein can be separated from milk by acidification to pH 4.6, by using proteolytic enzymes, or by microfiltration. Acidification of milk can be brought about by microbial fermentation of lactose to lactic acid or by the addition of organic (citric acid or lactic acid) or mineral acids (sulfuric acid, phosphoric acid, or hydrochloric acid). Sweet whey is derived from the manufacture of cheese or casein by rennet coagulation of milk at a pH of about 6.0–6.5. Typically, sweet whey results from the production of hard and semi-hard cheeses. Acid whey results from fermentation during fresh acid-coagulated cheese production, and from direct acidification of milk during casein and caseinate production. The resulting whey has a pH of about 4.6–5.0 (Bansal and Bhandari, 2016).

In the dairy industry, it is estimated that on average 9 L of whey result from each kg of cheese, which is classified as a large wastewater generator with a high pollutant load (Kushwaha *et al*., 2010; Bansal and Bhandari, 2016). Global production of this effluent is estimated between 180 to 190 million t $year^{-1}$, approximately 50% of which is processed or redirected to industrial processes; therefore, 90 million t $year^{-1}$ are discharged mainly in water sources around the world (Baldasso *et al*., 2011; Prazeres *et al.,* 2012; Bansal and Bhandari, 2016).

According to the Ministry of Agriculture and Rural Development of Colombia (MADR), production of "Campesino" or white cheese and double cream cheese for 2019 amounted to 1460 t and 1624 t, respectively, indicating that 24% of the milk formally collected in Colombia is used for cheese production (Gonzalez, 2021). As a result of this amount of cheese, approximately 27756 t of whey were obtained (Gómez and Sánchez, 2019). However, this value only considers what was generated by the formal industry, according to estimates by Muset and Castells (2017) the whey production in Colombia is 2.1 million t $year^{-1}$ and, between 70 to 80% of this volume is being discharged without adequate treatments.

Traditional practices for the use of whey are fertilization of soils by direct irrigation, animal feed, and spray drying (Bansal and Bhandari, 2016). Currently, other technologies have been developed for whey treatment, e.g., microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO), and diafiltration (DF), which allow the fractionation of its components and makes it possible to obtain concentrates rich in protein and lactose with high economic value in the market (Jelen, 2011). The ultrafiltration process selects macromolecules weighing between 1000 – 200000 Da from solvents and dissolved solutes, with pore sizes ranging from 1 to 100 nm. The feed flow is carried out at less than 1000 kPa, considering low pressure, compared to nanofiltration and reverse osmosis. The permeate obtained, also known as ultrafiltrate, contains water, lactose, soluble minerals, non-protein nitrogen, and water-soluble vitamins. On the other hand, protein, fat, and colloidal salts can be found in the retentate (Mistry and Maubois, 2017). Almécija *et al*. (2007) worked on whey proteins concentration and fractionation with ultrafiltration (UF) technology by testing the effect of feeding solution, pH between values of 4.2 and 7.3 in process efficiency. Yorgun *et al.* (2008) worked with Turkish white cheese whey, obtained by acid-enzymatic coagulation of milk, and UF, NF, and RO modules were tested to recover their different fractions. Baldasso *et al.* (2011) used mozzarella cheese whey to obtain a high purity protein concentrate using UF and DF, testing different cycles and volumes of water added in that technology. Barba *et al.* (2001) also demonstrated the effectiveness of UF and DF to obtain a high purity protein concentrate from whey with pH 4. Rektor and Vatai (2004) tested the efficiency of MF, UF, NF, and RO in mozzarella cheese whey fractionation in protein, lactose, and microorganism fractions. Butylina *et al.* (2006) obtained whey by enzymatic coagulation, prefiltered it before being treating it with UF and NF, and evaluating peptide loss when performing concentration

at different pH values. Besides, whey protein concentrate (WPC) is commonly used to increase the dietary protein in many novelty foods (Ranaweera *et al*., 2022). They can form different gel types depending on the balance between attractive and repulsive interactions (Domian and Mańko-Jurkowska, 2022). They are also used as an effective carrier and stabilizer of bioactive molecules and for improving the stability and antioxidant capacity of anthocyanins (Ji *et al*., 2022). An important part of Colombian whey is obtained as a result of the fresh curds manufacture, no bacteria added, by enzymatic coagulation of milk. As far as the authors know, no works have been found aimed at concentrating whey proteins using UF technology with whey from fresh regional cheeses (white cheese). Therefore, the aim of this study was to characterize whey and to evaluate the effect of filtration conditions, volumetric concentration factor (VCF), and transmembrane pressure (TMP) on protein retention, the yield of the UF process, and the properties of WPC. In addition, the determination of the best process conditions was carried out using multiple-response optimization, maximizing the desirability function to search for the maximum value of each independent variable.

MATERIALS AND METHODS

Physicochemical and compositional characterization of raw material and whey protein concentrate

The milk used to make white cheese was obtained from dairy farms that breed Holstein cows, it had an average protein content of 3%; fat, 3.6%; total solids, 12%. The animals are fed on Kikuyu grass pastures (*Pennicetum clandestinum*). Milk was obtained through automatic cow milking and was stored in refrigeration until it was collected by the processing company. Sweet whey resulting from fresh cheese preparation and enzymatically coagulated milk (white cheese) was supplied by Productos Lácteos Aura SA (Rionegro, Antioquia, Colombia). White cheese is traditional from Colombia, it is fresh, made by enzymatic coagulation without the addition of bacteria. It was made using whole milk, which was pasteurized at 75 °C for 15 s, then cooled to 35 °C, when the coagulating enzyme (Chimax, CHR Hansen, Denmark) is added. The curd was cut and stirred for 10 min, completely drained, and, finally, salt (sodium chloride) at 1.2% was added. The resulting mass was molded, pressed for 10 min, portioned, and packed to be stored under refrigeration (0-6 °C). The whey was filtered, clarified by centrifugation at 7200 rpm, and thermized at 63 °C. Total protein content was determined by the Kjeldahl method (AOAC 920.123, 1997); fat content by the Babcock method (AOAC 989.04, 1997); total solids by oven drying by forced air at 98-100 °C for 3 h (AOAC 925.23, 1997); acidity by titration with 0.1N NaOH (AOAC 947.05, 1997); pH with a potentiometer (AOAC 981.12, 1997); water activity (Aw) by dew point method in a thermo-hygrometer at 25 °C (AquaLab 3TE Series, Decagon Devices, Inc., Pullman, Washington, USA) (Grummer and Schoenfuss, 2011); the zeta potential using a Zetasizer light scattering kit (Malvern Panalytical); and it was used to measure the electrophoretic mobility distribution. The whey sample was injected into the measurement cell and placed in the equipment. The temperature was set at 25 °C and a voltage of 200 mV was applied. The analysis lasted approximately 10 min (Gbassi *et al*., 2012). The lactose content was quantified by HPLC using a 1200 series Agilent Technologies equipment (Santa Clara, CA, USA) with an Aminex HPX-87H ion exchange column (300×7.8 mm) (Bio-Rad) (Hercules, CA, USA); an Agilent Technologies 1200 refractive index detector H_2SO_4 0.008 N was used as mobile phase at a flow rate of 0.6 mL min^{-1} (AOAC 984.22, 1997); α-lactoalbumin and β-lactoglobulin content were determined by HPLC using a Shimadzu chromatograph (Kyoto, Japan) equipped with a diode array detector (DAD); and a Jupiter® column (5 µm C18 300 Å, 250×4.6 mm) (Phenomenex) (Torrance, CA, USA) was used with trifluoroacetic acid (TFA) and acetonitrile (ACN) as mobile phases (Elgar *et al.,* 2000). The flow rate was 1 mL min^{-1}. The column was adjusted in 80% solvent A (0.1% v/v, TFA in Milli-Q water) and, after sample injection, a 1 min isocratic period was applied; subsequently, a linear gradient series of 100% solvent B (0.09%, v/v, TFA, 90%, v/v acetonitrile in Milli-Q water) was applied as follows: 1-6 min 20 – 40% B; 6-16 min, 40-45% B; 16-19 min, 45-50% B; 19-20 min, 50% B; 20-23 min, 50-70% B; 23-24 min, 70-100% B. The column was adjusted again after 1 min at 100% B with a 2 min linear gradient to 20% B followed by a 3 min isocratic period.

Concentration of whey proteins using UF

Each experiment was done with 800 L of standardized sweet whey which was ultrafiltered (UF) in a pilot

plant (Perinox, Villarobledo, Spain) equipped with two polyethersulfones (PES) membranes in a spiral module (Koch Membrane System inc., Wilmington, Massachusetts, USA) with a capacity of 130 L h^{-1} of permeate flow and a molecular weight cut-off (MWCO) of 10 kDa. The VCF was between 5 and 18 and the TMP was between 2 and 5 bar. The VCF and TMP values used in the experimentation are shown in Table 1. The protein retention coefficient (Rp) was determined in Eq. (1) (Alkhatim *et al*., 1998; Vourch *et al.,* 2005; De Souza *et al.,* 2010); permeate flow (Jp) in Eq. (2); and yield (Y) in the protein's recovery (Nath *et al*., 2014) and protein concentrate content (AOAC 920.123, 1997) in Eq. (3). These variables have great importance in concentration process efficiency. All tests were carried out at a constant temperature of 48 °C, according to literature (Barba *et al.,* 2001; Butylina *et al.,* 2006; Bipasha *et al.,* 2016).

$$Rp = \left(1 - \frac{Cp}{Cr}\right) \times 100 \quad (1)$$

Where Rp is the protein retention coefficient, Cp is the solute in the permeate (kg), and Cr is the solute in the retentate (kg).

$$Jp\ -V/(A \times t) \quad (2)$$

Where Jp is the permeate flow (L m^{-2} h^{-1}), V is the volume of permeate (L), A is the effective filtration area (m^2), and t is the time (h).

$$Y = \frac{Vr \times Cr}{Vf \times Cf} \quad (3)$$

Where Y is yield, Vr is the retentate volume (L), Cr is the solute concentration in retentate (% w/w), Vf is the feed solution volume (L), and Cf is the solute concentration in feeding solution (% w/w).

Design and statistical analysis

A central composite orthogonal design was used to optimize the independent variables TMP and VCF in Rp, Jp, Y, and protein concentrate content in the protein concentration process by UF technology. The independent variables were selected from preliminary trials and equipment setups. An individual optimization for each independent variable was performed, and then, a multiple-response optimization maximizing the desirability function to search the maximum value of each independent variable. Data analysis was carried out using Statgraphics Centurion 16.1 (Statpint Technologies, INC).

RESULTS AND DISCUSSION

TMP had significant effects (P<0.05) on Jp as shown in Figure 1A because the Jp at a TMP of 2 bar did not exceed 37.5 L m^{-2} h^{-1}, and when TMP was 5 bar it was significantly higher, exceeding 41 L m^{-2} h^{-1}. Baldasso *et al*. (2011) reported that Jp is affected by concentration-polarization and fouling phenomena, which is based on the accumulation of a solute layer on the membrane's surface and pores, or interactions between the solutes and the membrane surface, respectively. This process increases with whey concentration (Nath *et al.,* 2014). Nevertheless, Jp is related to intrinsic membrane resistance (Rm) and the resistance due to fouling (Rf) (Barba *et al.,* 2000), which increase due to protein concentration and VCF (Baldasso *et al*., 2011). However, TMP was effective in increasing the permeate flux for the range studied, being a phenomenon that was previously reported (Iltchenco *et al*., 2018; Barba *et al*., 2000). TMP had no effect (P>0.05) on Rp (Figure 1B), which indicates that the membrane did not let escape the protein fractions as the pressure increased. Iltchenco *et al*. (2018) and Barba *et al*. (2000) reported that the pressure and the temperature did not affect these variables when 10 kDa MWCO membranes are used to concentrate whey proteins.

The VCF presented a significant effect (P<0.05) on the Rp; it had values between 0.6 and 0.73 (Figure 1B), and this was similar to the literature (Galanakis *et al*., 2014). Iltchenco *et al.* (2018) reported values between 0.68 and 0.84 using an MWCO between 100 to 1 kDa from polysulphone and polyethersulphone, and De Souza *et al.* (2010) reported 84%. With higher values of VCF, the protein concentrate content (%) in the permeate also increases, which is represented by peptides, free amino acids, and compounds consisting of non-protein nitrogen (Butylina *et al*., 2006). However, Barba *et al.* (2000) stated that native proteins, such as β-lactoglobulin and α-lactalbumin, might even permeate, and this phenomenon can increase due to higher protein concentration and higher osmotic pressure. The Rp indicates that size exclusion was the

dominant phenomenon during the separation process (Galanakis *et al.*, 2014) due to the molecular weight of whey proteins, which are higher than 14 kDa (Jelen, 2011), and the membrane nominal pore size, which is 10 kDa.

There is efficient solute retention in the protein concentration process by UF when using a PES membrane with MWCO of 10 kDa. However, small fractions as peptides and even native proteins such as β-lactoglobulin and α-lactalbumin (Barba *et al.*, 2000) can go through the membrane. The yield was not affected by the TMP and VCF factors (P>0.05) and was within the range between 0.51 to 0.84 (Figure 1C). This values agree with the literature, for instance, Nath *et al.* (2014) reported a yield of 0.6-0.85 for separation of individual whey protein fractions and lactose from casein whey by a cascade of different molecular weight cut-off (MWCO) cross-flow ultrafiltration (UF) membranes; Kukučka and Kukučka (2013) reported a yield of 0.4-0.65 for separation of protein from sweet whey using polysulfone ultrafiltration membranes designed for commercial water treatment; and Marella *et al.* (2011) reported a higher yield when compared to that of production of α-lactalbumin (α-LA)–enriched whey protein concentrate (WPC) with a range of 0.35-0.45.The use of TMP higher than 2.06 bar helps in the permeation of some high-molecular-weight molecules like proteins (Nath *et al.*, 2014).

The optimization process was carried out to maximize the desirability parameter taking into account Rp, Jp, and the protein concentrate content (%). In the optimization options, a higher impact was given to the protein concentrate content (%) (5 points) because the interest was to have a concentrate with a maximum protein content to be used in cheese making, whereas Rp and Jp had an impact of 3 points each. The desirability resulted in 0.61, and the optimized values for TMP and VCF were 2 bar and 18, respectively. The Jp, Rp, and protein concentrate content (%) were 42.97 L m^{-2} h^{-1}, 0.54, and 9.08%, respectively.

There were significant differences (P<0.05) between whey and WPC in the values of lactose, protein, acidity, total solids, fat, zeta potential, β-lactoglobulin, and α-lactalbumin (Table 2). The WPC is available in various

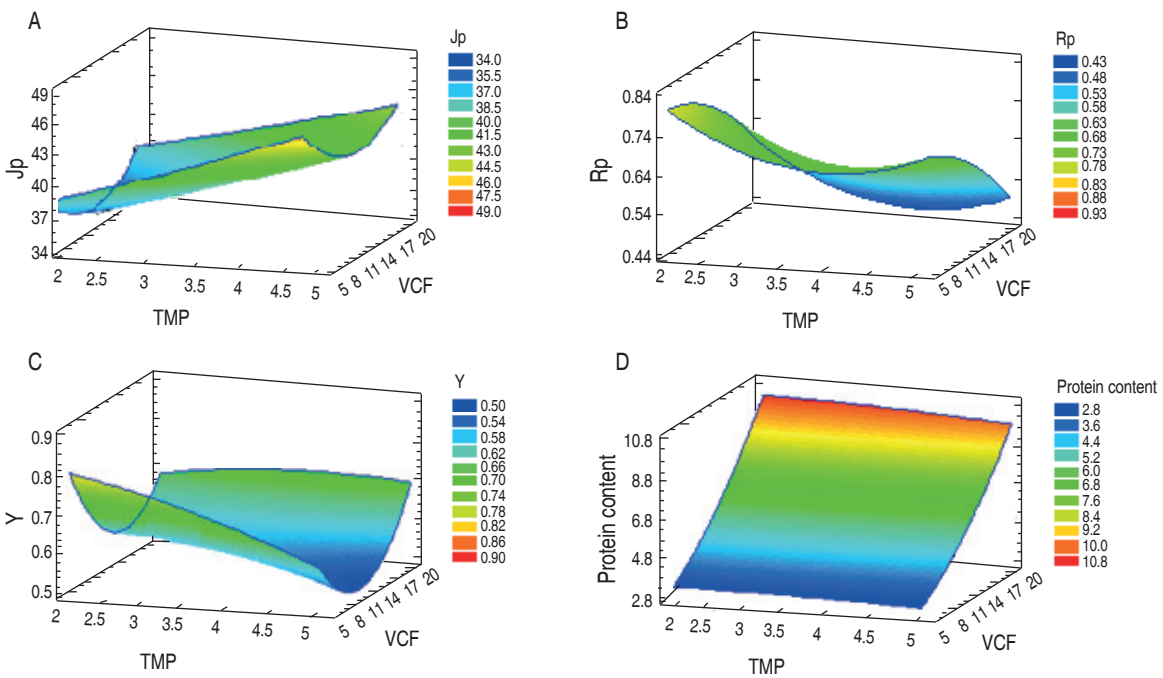

Figure 1. Effect of TMP and VCF on (A) Jp: Permeate flow (L m^{-2} h^{-1}); (B) Rp: Protein retention coefficient; (C) Y: yield; and (D) protein concentrate content (%).

protein concentration levels such as 34% (WPC34), 45, 50, or 80%, and this denomination is given by the relationship between the protein and total solids content (Gangurde *et al.,* 2011). The proteins content in the WPC was 8.27% (Figure 1D) and the total solids was 18.21%, then the protein represented 45% of the total solids. Therefore, the WPC was classified as a WPC 45 from sweet whey, which is an interesting additive for the food industry because it is rich in proteins, minerals, and lactose and can influence the structure, appearance, texture, viscosity, mouthfeel, or flavor retention of the dairy food products (Królczyk *et al.*, 2016). Protein and fat were concentrated by UF, however, a less stable system was obtained according to zeta potential data. Two types of whey can be made as a result of the separation of caseins from milk, acid whey (pH<5), and sweet whey (pH = 6-7). The previous data is in agreement with the values observed in this study (Panesar *et al.*, 2007; Parra, 2009).

Table 1. Effect of TMP and VCF on Rp, Y, Jp, and protein concentrate content (%).

TMP (bar)	VCF	Jp (L m^{-2} h^{-1})	Rp	Y	Protein concentrate content (%)
5	5	44.6	0.72	0.63	2.84
3.5	5	43.9	0.73	0.66	2.96
5	18	41.4	0.60	0.59	8.46
3.5	11.5	37.1	0.61	0.59	5.39
2	11.5	35.8	0.81	0.54	5.61
5	11.5	44.8	0.60	0.51	5.23
2	18	36.4	0.62	0.62	8.94
2	5	37.5	0.73	0.84	2.95
3.5	18	41.2	0.45	0.64	9.21
3.5	11.5	35.8	0.63	0.60	5.17

Table 2. Characteristics of white cheese whey and whey protein concentrate.

Characteristic	Whey protein concentrate (WPC)	White cheese whey	Sweet whey*
Lactose (%)	3.90±0.1a	4.60±0.2 b	4.6-5.2
Protein (%)	8.27±0.5 a	0.85±0.03 b	0.6-1.0
pH	6.24±0.3 a	6.52±0.3 b	6-7
Acidity (%)	0.43±0.02 a	0.08±0.01 b	0.2
Total solids (%)	18.21±0.4 a	6.98±0.4 b	6.3-7.0
Fat (%)	2.41±0.2 a	0.08±0.01 b	
Zeta potential (mV)	-23.44±0.7 a	-31.33±0.8 b	
β-lactoglobulin (%)	4.71±0.3 a	0.40±0.01 b	
α-lactalbumin (%)	1.38±0.1 a	0.153±0.01 b	

* Taken from Jelen (2011).
Values are means ± the standard deviation of the measurements. Different superscripts in the same row indicate significant differences (P<0.05).

According to Jelen (2011), the whey used in this study had a lower content of calcium and acidity (percentage of lactic acid), which was a phenomenon explained by the cheese-making technology that generated sweet whey. In other countries, cheeses coagulated enzymatically involve using bacteria (Cheddar, Gouda, and Edam

cheeses) and act by slightly fermenting the milk. This process differs in Colombia because cheeses are a result of enzymatic coagulation of milk without adding bacteria (fresh curds). In addition, colloidal calcium is solubilized at lower pH values and higher acidity conditions (Panesar *et al.*, 2007), thus explaining the lower calcium content of sweet whey found with respect to the literature (Jelen, 2011) (Table 1). According to Carvalho *et al.* (2013), bovine whey typically contains, on a dry basis, between 70-80% lactose, 10-12% protein, and between 8-20% minerals, as well as presenting a pH of 6.6 (De Wit, 2001; Muro-Urista *et al*., 2010). These values are similar to the results shown in this study, being 66% lactose, 12% protein, and 7.6% minerals. β-lactoglobulin and α-lactalbumin contents are similar to those reported by other authors at 0.3 and 0.1%, respectively (Madureira *et al.,* 2010, Almécija *et al.,* 2007).

Concentrate showed significant differences (P<0.05) with respect to whey without concentrating, lactose content, protein, pH, total solids, fat, zeta potential, β-lactoglobulin, and α-lactalbumin. Whey contains between 10 and 12% of protein (bs) with a size between 8.6 to 150 kDa (Carvalho *et al.,* 2013). Therefore, when using a UF membrane with a molecular cut-off size of 10 kDa, a large part of these can be retained, thus explaining the increase of total and individual protein content. This same principle applies to fatty matter, which is retained by this membrane. The WPC had an important protein concentration up to WPC45, indicating that the UF objective was achieved by removing water and other small molecules (Arunkumar and Etzel, 2015). The zeta potential was closer to zero in the concentrate indicating that it is a less stable system than whey because the higher concentration of protein in concentrate can result in more collisions between molecules, generating flocculation (Kaewkannetra *et al.,* 2009). Furthermore, the acidity was higher in the concentrate because the concentration temperature was 43 °C and thermophile bacteria could grow and produce lactic acid, thus lowering the pH value from 6.5 to 6.2 as reported by De Wit (2001) for WPC.

CONCLUSIONS

UF is a suitable technology for sweet whey concentration obtained from the manufacture of fresh enzymatically coagulated cheeses without adding bacteria, a typical process of Colombian cheeses. From this whey, it is possible to obtain a WPC of 45% protein content, which can be used to develop ingredients for food industry. WPC must be used quickly because it tends to generate whey protein flocculation. Under the proposed process conditions, the optimal point for protein concentration, determining the maximization of protein content, was at a TMP of 2 bar and VCF of 18.

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Improvement of productive and metabolic indicators of broiler by the application of *Lippia origanoides* essential oil in an *in vivo* intestinal inflammation model

Mejoramiento de los indicadores productivos y metabólicos de los pollos de engorde mediante la aplicación de aceite esencial de *Lippia origanoides* en un modelo de inflamación intestinal *in vivo*

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ABSTRACT

Broiler meat is the animal origin protein source with the highest nutritional quality, growth, and projection in the last decade. However, the presence of traces of antimicrobials in the final products, mainly due to the indiscriminate addition of antibiotics (prophylactic use) as growth-promoters antibiotics (GPA) has raised major concerns. In the search for viable alternatives, natural additives such as essential oils appear, among which the oregano (OEO-*Lippia origanoides*) stands out. It has been used with success at an industrial level and its antimicrobial properties are well known. The current study aimed to evaluate the zootechnical parameters (accumulated weight gain, feed conversion, and feed efficiency) and blood metabolites (Alanine aminotransferase, glucose, cholesterol, triglycerides, creatinine, phosphorous, calcium) in broilers adding OEO into their diet in an *in vivo* inflammation model achieved by adding Lipopolysaccharides (LPS) from *E. coli*. 1200 animals were distributed in six diets: D1: basal diet (control) or balanced commercial diet without the addition of GPA, OEO, and LPS; D2: D1+150 ppm GPA (Avilamycin); D3: D1+150 ppm OEO (*Lippia Origanoides*); D4: D1+1.0 ppm LPS; D5: D1+1.0 ppm LPS+150 ppm GPA; D6: D1+1.0 ppm LPS+150 ppm OEO. The trial was carried out under a randomized block design. OEO-*Lippia origanoides* improved the zootechnical and metabolic variables (P<0.05) of birds in the face of *in vivo* inflammation at different ages; furthermore, it improved the animal response and the metabolic conditions of the animals under study. The zootechnical and blood metabolites results at different ages evidenced the technical feasibility of OEO as a nutritional growth promoter.

Keywords:
Blood metabolites
Broilers
Lippia origanoides
Zootechnical parameters

RESUMEN

La carne de pollo es la fuente de proteína de origen animal con mayor calidad nutricional, crecimiento y proyección en la última década. Sin embargo, la presencia de trazas de antimicrobianos en los productos finales, debido a la adición indiscriminada de antibióticos (uso profiláctico) como antibióticos promotores del crecimiento (GPA) ha suscitado gran preocupación. Sin embargo, es necesario profundizar en las propiedades del OEO frente a un proceso infeccioso bacteriano (lipopolisacárido-LPS) e inflamatorio a nivel intestinal y su relación con parámetros productivos. El presente estudio tuvo como objetivo evaluar los parámetros zootécnicos (ganancia de peso acumulada, conversión y eficiencia alimenticia) y los metabolitos sanguíneos (Alanina aminotransferasa, glucosa, colestreol, triglicéridos, creatinina, fósforo, calcio) en pollos de engorde añadiendo a su dieta OEO en un modelo de inflamación *in vivo* conseguido mediante la adición de LPS de *E. coli*. Se distribuyeron 1200 animales en seis dietas D1: dieta basal (control) o dieta comercial equilibrada sin la adición de GPA, OEO y LPS; D2: D1+150 ppm GPA (Avilamicina); D3: D1+150 ppm OEO (*Lippia Origanoides*); D4: D1+1,0 ppm LPS; D5: D1+1,0 ppm LPS+50 ppm GPA; D6: D1+1,0 ppm LPS+150 ppm OEO. El estudio se realizó bajo un diseño de bloques al azar. AEO-*Lippia origanoides* mejoró las variables zootécnicas y metabólicas (P<0,05) de aves ante una inflamación *in vivo* a diferentes edades; además, mejoró la respuesta animal y las condiciones metabólicas de los animales en estudio. Los resultados zootécnicos y de metabolitos sanguíneos a diferentes edades evidenciaron la viabilidad técnica del AEO como promotor nutricional de crecimiento.

Palabras clave:
Metabolitos sanguíneos
Pollos
Lippia origanoides
Parámetros zootécnicos

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Poultry meat production has grown substantially, doubling from the year 2000 to 2017, and achieving more open and competitive export markets worldwide. Additionally, more than 20% of world poultry production is in Latin America (Naciones Unidas, 2019). The growth of this production is due to the high demand for low-cost, high-quality animal protein, which, in turn, contributes to food security (FAO, 2018) and helps fight hunger of 795 million people around the world (98% live in developing countries) and malnutrition undergone by 12.9% of this population (FAO *et al*., 2018). For all the above mentioned, in Colombia, intensive poultry meat production has increased, becoming the most consumed meat in recent years (32.1 kg per person), exceeding bovine and pig origin product consumption (FENAVI, 2020).

The increase in the productive and economic performance rates of broilers under intensive conditions has triggered animal stress, increasing the deficiencies and imbalances in the composition of their intestinal microbiota. This has led to the appearance of frequent digestive disorders and the decrease in natural resistance to colonization by pathogenic microorganisms and their toxins (mainly lipopolysaccharide-LPS), causing chronic inflammatory processes at the intestinal level (Blajman *et al.,* 2015).

Lipopolysaccharide is a bacterial endotoxin found predominantly on the outer membrane of gram-negative bacteria and is a potent stimulator of the immune response (Wellington *et al*., 2020). Therefore, the dietary addition of antimicrobial compounds is of great importance in the production process, mainly by reducing pathogenic bacterial populations and increasing productive parameters (Valenzuela-Grijalva *et al*., 2017). Nevertheless, given the increase in poultry meat production and consumption, there is a need for strict controls on the quality and safety of products that reach the final consumer.

The GPA use has become a public health concern due to the presence of traces in meat and the appearance of multi-resistant bacterial strains. It has led to the limited or prohibited non-therapeutic use (prophylactic and metaphylactic) of some antibiotics in different countries (García-Hernández and García-Curbelo, 2015). Additionally, additives must be recognized as GRAS (Generally Recognized As Safe) by the United States Food and Drug Administration (FDA) (Ciro *et al*., 2014). The essential oil of oregano (OEO) is extracted from the species *Lippia origanoides* belongs to the Verbenaceae family and has received the highest importance because it is an essential component of the Colombian biodiversity. In addition, it has the typical oregano chemotypes, that is, thymol predominates in its composition (between 67.2 and 78.7%), and there are low concentrations of carvacrol (between 0.9 and 1.2%) (Ortiz *et al*., 2017). Therefore, OEO is projected as a nutritional growth promoter with immunological benefits in broilers and GPA replacement (Madrid-Garcés *et al*., 2017).

Since most studies have evaluated different zootechnical, metabolic, and clinical parameters in broilers fed with OEO in optimal health conditions (Madrid-Garcés *et al*., 2018a), it is important to study the effect of this natural additive in intestinal inflammatory conditions induced by the administration of LPS of *E. coli*. This is one of the *in vivo* models most used in studies of acute infectious processes, as it has highly reproducible actions in different species and lacks the side effects associated with chronic bacterial infections (Ciro *et al*., 2014). Accordingly, this work aimed to evaluate the zootechnical parameters and blood metabolites of broilers fed with the addition of oregano essential oil (*Lippia Origanoides*) in an *in vivo* intestinal inflammation model (by adding LPS from *E. coli*).

MATERIALS AND METHODS

Ethical considerations. All the experimental procedures were carried out according to the guidelines proposed by "The International Guiding Principles for Biomedical Research Involving Animals" (Iclas, 2012; Leary *et al*., 2013). The Ethics Committee of Universidad Nacional de Colombia, Medellín campus endorsed this study through CEMED-013 of May 4, 2016.

Study site. The fieldwork was carried out at the San Pablo Agrarian Station that belongs to Universidad Nacional de Colombia, Medellín Campus, located in the municipality of Rionegro (Antioquia), in the zone called "El Tablacito" located at 2100 masl. The zone has a temperature between 12 and 18 °C, corresponding to a low montane very humid forest life zone (bmh-MB).

Animals. 1200 one-day-old male broilers of the ROSS308 genetic line from a commercial incubator were analyzed. These were housed in floor pens to recreate the density conditions of a commercial production, of which 420 were randomly selected (euthanized) for sampling in this experiment. Broilers rearing was carried out following commercial procedures employed on an experimental farm (Aviagen, 2017). The experimental period lasted 42 days.

Sanitary management. Before receiving the one-day-old broilers from a commercial incubator already vaccinated against Marek, Newcastle, and Gumboro diseases, the housing preparation procedures were developed as described below: washing, cleaning, and disinfection of the pens, curtains, feeders, and drinkers. Further, rodent and insect control was carried out with products obtained from commercial houses. The brooders were turned on 5 h before the arrival of the animals to preheat the pens up to a temperature of 32 °C for the moment when the animals were received (Aviagen, 2017).

Diets. A balanced commercial diet without the addition of antibiotics, essential oils, or LPS was used as a basal diet. In this basal diet, the growth-promoting antibiotic (GPA) Avilamycin, essential oil of oregano (OEO-*Lippia Origanoides*), and the endotoxin lipopolysaccharide (LPS) of *E. coli* as an *in vivo* intestinal inflammation model (Ciro *et al*., 2014) were added to the basal diet establishing the final diets (treatments), as follows:

- Diet 1 (D1): Basal Diet (control) or balanced commercial diet without the addition of GPA, OEO, and LPS.
- Diet 2 (D2): D1 with the addition of a GPA (Avilamycin, 150 ppm).
- Diet 3 (D3): D1 with the addition of OEO (150 ppm).
- Diet 4 (D4): D1 with the addition of 1.0 µg of LPS g^{-1} of food (1 ppm).
- Diet 5 (D5): D1+1.0 µg of LPS g^{-1} of food (1 ppm) and a GPA (Avilamycin, 150 ppm).
- Diet 6 (D6): D1 with the addition of 1.0 µg of LPS g^{-1} of feed (1 ppm) and OEO (150 ppm).

The basal diet was established for two growth stages and met the minimum nutritional requirements for the genetic line (Aviagen, 2017) (Table 1). The feed given during the first 21 days of the experiment was called "Start." For the following 22 days, the broilers were fed with the diet called "Finalization". The feed was free of antibiotics, except for diets D2 and D5, where Avilamycin was used as a GPA, as it is done commercially. OEO was added to the pre-mix at an effective concentration of 150 ppm and used as a phytobiotic for diets D3 and D6, according to Madrid-Garcés *et al*. (2018a). The addition of 1.0 µg of LPS g^{-1} of feed (1 ppm) (LPS *E. coli,* serotype 0111: B4 Sigma-Aldrich, Sigma-Aldrich St Louis, MO, USA) was used in the study to generate the inflammation process *in vivo* (Ciro *et al*., 2014) in diets D4, D5, and D6.

Table 1. Nutritional contribution of the basal diet (BD), designed in two stages: start and finalization

Nutrients	Start value	Finalization value
Weight (kg)	1.000	1.000
Moisture (%)	10.926	10.845
Metabolizable energy (birds) (kCAL kg^{-1})	3152.165	3299.259
Crude protein (%)	21.474	19.976
Fat (%)	8.301	10.213
N-free extract (NFE) (%)	49.673	50.195
Crude fiber (%)	2.927	2.801
Ashes (%)	6.108	5.379
Calcium (%)	0.997	0.832
Available phosphorus (%)	0.418	0.360
Total phosphorus (%)	0.648	0.580
Electrolyte balance(mEq kg^{-1})	216.164	191.553

Animal performance parameters. Within the performance parameters evaluated, accumulated weight gain (AWG- Indicates how much weight an animal gains by sampling age), feed conversion (FC), and feed efficiency (FE) were selected, according to Li *et al*. (2019). The parameters were evaluated on days 21 and 42. AWG, FC, and FE were calculated by parameterization of the following equations:

$$AWG = \text{Final Weight} - \text{Initial Weight}$$

$$FC = \frac{\text{Food consumed}}{\text{Weight gained}}$$

$$FE = \frac{100}{FC}$$

Good euthanasia practices and sampling. All birds were sacrificed 2.5 h after their last meal. Animals were sedated by inhalations with Nitrox® and subsequently, euthanized humanely by inhalation with carbon dioxide for 3 min (Madrid-Garcés *et al*., 2018a). During the experimental phase, 420 animals were euthanized on days 1, 7, 14, 21, 28, 35, and 42, 60 animals per day (10 animals per treatment). A blood sample was taken from the aorta vein of each animal. For this analysis, 5 mL of blood per bird were taken on days 1, 21, and 42. Using a 25-mm needle, a cardiac puncture was performed, specifically in the left ventricle, for the extraction of blood in a test tube with an anticoagulant. Each of the samples was kept cold at 4 °C while being transported to the laboratory, where they were centrifuged at 1600 rpm for 10 min to separate the plasma into aliquots and then be analyzed (Maya-Ortega *et al.,* 2021).

Quantification of blood metabolites. The metabolites evaluated were alanine aminotransferase, glucose, cholesterol, triglycerides, creatinine, calcium, and phosphorus. The quantification of the analytes in serum was carried out using the commercial slides VITROS® kits (Ortho Clinical Diagnostics, USA) specific for each analyte, following the manufacturer's instructions. A VITROS® DT-60II biochemical analyzer (Ortho Clinical Diagnostics, USA) was used to measure optical densities (Madrid-Garcés *et al*., 2018b).

Statistical analysis. For the analysis of the productive variables, a factorial model (6×2) was carried out, with six diets, two measurements days, and 10 replicates (each pen was an experimental unit). Each of the animals was randomly assigned to one of the six diets. The statistical analysis was performed according to the Proc Mixed procedure of the SAS® program version 14.3 software (SAS, 2017).

For the analysis of the different metabolites, a factorial model was performed, where the animals were randomized to one of the different diets (six), and each treatment (6 diets × 7 ages) had a total of 10 repetitions. The assumption of normality was analyzed using the Shapiro-Wilk test. The assumption of homoscedasticity was analyzed using the Bartlett test. And independence was guaranteed through good experimental practices. The multiple pair-wise comparisons were analyzed using a Tukey test (honestly significant difference-HSD) to establish the possible differences between groups. P-value significance was established at $P<0.05$.

RESULTS AND DISCUSSION

In general, the animals that consumed the different diets showed good health, without adverse symptoms or signs of disease that caused their withdrawal or euthanasia, or both, before the sampling periods. Additionally, the animals consumed the daily feed ration adjusted to the management guide for the Ross308 genetic line. In this experiment, no statistical interaction was found between different diets, evaluation days, and weaning age for any of the response variables in the study, so it was not necessary to analyze and disaggregate these factors independently.

Productive parameters

Accumulated weight gain (AWG). The accumulated weight gain (Table 2) did not show a statistically significant difference ($P>0.05$) at 21 days of age in the diets without the addition of LPS, obtaining D3 the numerically highest value in the different variables evaluated; furthermore, there was no difference ($P>0.05$) between the diets with the addition of LPS, where a reduction in productive performance was observed. When comparing the diets with LPS and those without the addition of LPS, the LPS-free diets presented better values ($P<0.05$). On day 42, a significant statistical difference was found between the

diets, and D3 showed the best values (P<0.05). However, there were no differences (P>0.05) between D5 and D6. A statistically significant difference (P<0.05) was evidenced between day 21 (completion of the growing phase) and day 42 (completion of the fattening phase) for the AWG variable within each diet under study.

Table 2. Zootechnical parameters in broilers fed with the addition of oregano essential oil **(***Lippia origanoides*) in an *in vivo* intestinal inflammation model.

Indicators	Day	D1 control	D2 GPA	D3 OEO	D4 LPS	D5 LPS+GPA	D6 LPS+OEO	P Value
AWG (g)	21	595.5 a, y	598.0 a, y	601.6 a, y	568.5 b, y	574.3 b, y	579.7 b, y	0.00066
	42	2808.5 a, z	2887.5 b, z	2910.5 c, z	2668.0 d, z	2736.5 e, z	2737.0 e, z	0.00054
FC	21	1.68 ab	1.67 ab, y	1.58 a, y	1.73 b, y	1.71 b, y	1.71 b, y	0.00068
	42	1.56 ac	1.46 ab, z	1.43 b, z	1.63 c, z	1.59 c, z	1.59 c, z	0.00058
FE	21	59.5 ac, y	59.8 ab, y	64.9 b, y	57.8 c, y	58.4 c, y	58.4 c, y	0.00073
	42	64.9 a, z	68.4 b, z	69.9 b, z	61.3 c, z	62.9 d, z	62.9 d, z	0.00062

AWG: daily weight gain. FC: feed conversion. FE: feed efficiency, a, b, c, d, e within the same row, means with the same letters are not statistically different (P<0.05), y, z within the same column, means with the same letters are not statistically different (P<0.05).

Feed conversion (FC). Feed conversion on day 21 showed statistically significant differences (P<0.05) between D3 and the diets with LPS (D4, D5, and D6), but no statistically significant differences (P>0.05) were recorded with D1 and D2 (Table 2). Between D1 and D2, there was no difference (P>0.05), and further, between the diets with LPS, no difference was observed (P>0.05). By day 42, a significant statistical difference was found between the diets without LPS, and D3 obtained the best results. Nevertheless, there was no difference between D3 and D2, and between the latter and D1 (P>0.05). There was no difference (P>0.05) between the diets with the addition of LPS (D4, D5, and D6). Nonetheless, these diets did not show differences with D1 (P>0.05) (Table 2). A statistically significant difference (P<0.05) was evidenced between day 21 (completion of the growing stage) and day 42 (completion of the fattening stage) for FC within each of the studied diets, finding differences in productive performance in the two stages.

Feed efficiency (FE). FE for day 21 obtained a significant statistical difference (P <0.05) between D3 and the other diets, except for D2, which also presented statistical differences (P<0.05) with D4, D5, and D6. However, these last diets did not show differences with D1 (P>0.05). On day 42, there was no significant statistical difference (P>0.05) between D2 and D3, and neither between D5, and D6. Nonetheless, a statistical difference (P<0.05) was found between D1 and the other diets. Further, a statistically significant difference (P<0.05) was evidenced between day 21 (completion of the growing stage) and day 42 (completion of the fattening stage) for FE within each diet under study. With the data obtained in this work, most results showed a positive effect on the zootechnical parameters when using GPA (D2) and OEO (D3), when compared with the basal diet (D1). These results corroborate what was found in other studies that compared different levels of OEO inclusion with a basal diet and another diet with Zinc Bacitracin as a GPA (Madrid-Garcés *et al*., 2017). The addition of LPS (D4) harmed the zootechnical parameters when compared with those obtained with the basal diet (D1) and with the diets that had GPA and OEO; however, in the diets that were given together with LPS+ GPA (D5) and LPS+OEO (D6), there was an improvement in the values, without reaching the values found with the basal diets (D1, D2, and D3). The values found on day 21 are similar to those reported by Trómpiz *et al*. (2011); these authors evaluated different diets on the fattening parameters of female chicks in their raising phase.

Concerning feed conversion (FC), average results similar to those found by Lázaro *et al.* (2017) were obtained on day 21, but very dissimilar from day 42. A study by Miranda-López *et al.* (2007) reported mean values for

day 21 similar to those of this study. Karimzadeh *et al.* (2017) reported values between 1.61 and 1.77 for the FC indicator, values very similar to those found in the present study. Furthermore, they reported AWG (kg) values between 2.186 and 2.419 on day 42, very different from the results obtained in the current study (between 2.668 for D4 and 2.910 for D3). Another study reported FC values between 1.583 and 1.655 (Broch *et al*., 2017).

Blood metabolites

Alanine aminotransferase (ALT). ALT obtained average values per day of age from 3.13 to 43.1 (U L^{-1}), presenting the highest values on day 21 of age (P<0.05). In the average values per diet, significant statistical differences were found for D2 with the other diets (P<0.05), except for D3 and D1(Table 3). Differences were found between D4 with D5 and D6, but no differences were found between the last two (P>0.05). Moreover, no statistically significant difference (P>0.05) was observed between D1 with D2 and D3. ALT results provide specific information about liver problems in an organism. In animals with fatty liver, ALT levels are usually higher (Alegría *et al.,* 2015). Other studies in broilers have reported values between 17.1 and 21.6 mg dL^{-1} (Bueno *et al.,* 2017), and between 10.84 and 17.28 mg dL^{-1} (Broch *et al*., 2017). The addition of GPA (D2) and OEO (D3) was shown to positively affect the ALT concentration in serum, reporting better values from the liver health standpoint. LPS had a negative effect on the ALT concentration, where the addition of GPA (D5) and OEO (D6) was done, having a mitigating effect on the damage generated by the addition of LPS. The negative effect of LPS may be explained by the metabolic demand of the body to generate an inflammatory response in the presence of a molecular pattern associated with a pathogen in the intestine.

Table 3. Alanine transferase (ALT, U L^{-1}) in blood serum of broilers fed with the addition of oregano essential oil (*Lippia origanoides*) in an *in vivo* intestinal inflammation model.

Day	Means	D1 control	D2 GPA	D3 OEO	D4 LPS	D5 LPS+GPA	D6 LPS+OEO	P value
1	31.3 t	3.13 t	3.14 tx	3.12 t	3.13 t	3.14 t	3.13 t	0.087
7	14.2 u	11.5 a, uy	9.80 a, ux	10.3 a, uy	21.7 b, u	15.1 c, u	16.7 c, u	0.00239
14	25.2 v	16.3 ac, vy	12.7 bc, u	14.6 c, vy	46.4 d, v	29.6 e, v	31.5 e, v	0.00014
21	43.1 w	38.2 a, w	25.6 b, v	30.6 c, w	59.7 d, w	49.2 e, w	55.3 f, w	0.00032
28	33.9 x	25.8 a, x	16.4 b, w	18.3 b, x	55.3 c, x	42.5 d, x	45.8 d, x	0.00026
35	19.6 y	14.9 ac, y	10.9 bc, u	13.6 c, y	29.9 d, y	21.9 e, y	26.2 f, y	0.00031
42	11.1 u	8.50 a, z	6.70 a, x	7.20 a, z	18.2 b, u	12.8 c, u	13.2 c, u	0.00028
	means	16.9 ac	12.2 bc	13.9 c	33.4 d	24.9 e	27.4 e	0.00029

Glucose. The average glucose values by age ranged from 266.6 mg dL^{-1} (day 7) to 316.3 mg dL^{-1} (day 42), where no differences were observed between the values of days 1 and 7 (P<0.05). The mean glucose values per diet ranged from 269 mg dL^{-1} (D4) to 300.4 mg dL^{-1} (D2) for the different diets (P<0.05). Significant statistical differences were found in the averages of the different diets, except between D2 and D3, which presented the highest values (Table 4). Glucose is one of the most critical blood metabolites as it is the basic metabolic fuel during the normal nutrition period of monogastric animals, and in turn, it is essential for the maintenance and continuous contribution to animal metabolism. Increases in glucose levels in fattening animals are related to increases in energy metabolism (Cunninghan, 2013). The data reported in this work differ from those found by Lázaro *et al.* (2017), being those of the current study substantially higher for all ages. The reason could be associated with the fact that the diet of the study mentioned above had lower metabolizable energy in the various stages. In a previous study by Madrid-Garcés *et al.* (2017), similar but lower values were reported explaining these results by the lower energy levels used in a multistage diet. Another study that evaluated the effect of age and cyclical heat stress on

the biochemical profile of broiler serum reported values between 248.4 and 282.7 mg dL^{-1} (Bueno *et al*., 2017), and between 199.76 and 217.20 mg dL^{-1} (Broch *et al.,* 2017). In general, the data were consistent, and the negative effect of LPS on this variable was evidenced due to the imbalance in homeostasis attributed to the induced inflammatory process. However, a positive effect was observed in the addition of GPA or OEO in the presence of LPS as an *in vivo* inflammation model. At 21 and 28 days of age, the best behavior was found in the diets with the addition of GPA and OEO. Both GPA and OEO showed a positive effect on intestinal inflammation.

Table 4. Glucose (mg dL^{-1}) in blood serum of broilers fed with the addition of oregano essential oil (*Lippia origanoides*) in an *in vivo* intestinal inflammation model.

Day	Means	D1 control	D2 GPA	D3 OEO	D4 LPS	D5 LPS+GPA	D6 LPS+OEO	P value
1	267.1 t	266.7 t	267.3 t	266.8 t	267.6 t	267.1 t	266.9 t	0.261
7	266.6 t	272.0 a, u	278.5 b, u	276.5 b, u	248.5 c, u	265.5 d, t	258.5 e, u	0.0035
14	275.9 u	280.5 a, v	292.0 b, v	288.8 c, v	255.0 d, v	271.5 e, u	268.5 e, t	0.0042
21	282.2 v	287.3 a, w	292.8 b, v	291.7 b, v	265.0 c, t	280.7 d, v	276.5 e, v	0.0046
28	294.4 w	298.0 a, x	316.5 b, w	308.5 c, w	276.0 d, w	286.0 e, w	280.0 f, w	0.0040
35	303.2 x	310.2 a, y	318.5 b, w	314.0 c, x	282.4 d, x	289.0 e, w	286.6 e, x	0.0052
42	316.3 y	321.5 a, z	337.5 b, x	336.5 b, y	291.0 c, y	307.0 d, x	304.5 d, y	0.0039
	means	291.2 a	300.4 b	297.5 b	269.0 c	281.0 d	275.7 e	0.0032

a, b, c, d, e eithin the same row, means with the same letters are not statistically different (P<0.05). t, u, w, x, y, z within the same column, means with the same letters are not statistically different (P<0.05).

Cholesterol. For the variable blood cholesterol, mean values were found for the different ages from 131.8 mg dL^{-1} (Day 1) to 193.1 mg dL^{-1} (Day 21), with statistically significant differences between all the ages assessed (P<0.05). Day 21 presented the highest values in each of the diets evaluated. For the different diets, values from 150.8 mg dL^{-1} (D2) to 167.2 mg dL^{-1} (D4) were found with statistically significant differences between all diets, except between D5 and D6, which D4 recorded the highest values (Table 5). Lázaro *et al*. (2017) found cholesterol values between 105 and 121 mg dL^{-1}, that is, lower than those reported in the current study. Regarding glucose, the differences may be associated with the use of various commercial diagnostic kits. Karimzadeh *et al*. (2017) reported values between 105 and 140 mg dL^{-1}. Bueno *et al.* (2017) observed values between 102.9 and 154.8 mg dL^{-1}, and Broch *et al.* (2017) found values between 104.32 and 117.20 mg dL^{-1}. Diets with the addition of LPS (inflammation model) were shown to have a negative effect, increasing blood cholesterol levels; nevertheless, the addition of GPA and OEO showed a positive effect on the inflammation model, without finding a statistically significant difference between the two treatments (D5 and D6).

Triglycerides. For triglycerides, mean values were found for the different ages from 128.1 mg dL^{-1} (Day 1) to 203.1 mg dL^{-1} (Day 21), observing a statistically significant difference between all the days (ages) evaluated (P< 0.05). For the average values of triglycerides in the different diets, values from 156.7 mg dL^{-1} (D2) to 186.4 mg dL^{-1} (D4) were found, and statistical differences between all diets (P<0.05), except between D2 and D3 (lowest values), and between D5 and D6 (P>0.05) were recorded (Table 6). Lázaro *et al*. (2017) found values between 85 and 97 mg dL^{-1}, which are relatively lower than those found in the current study. Meanwhile, Karimzadeh *et al.* (2017) reported values between 22.3 and 46.9 mg dL^{-1} and Bueno *et al*. (2017) between 30 and 33.7 mg dL^{-1}. The difference in values may be associated with the energy in the diet and the ingredients used for either extract component. The addition of LPS showed to have an effect of increasing blood triglycerides, while the diets with GPA and OEO had a positive effect since they

Table 5. Cholesterol (mg dL^{-1}) in blood serum of broilers fed with the addition of oregano essential oil (*Lippia origanoides*) in an *in vivo* intestinal inflammation model.

Day	Means	D1 control	D2 GPA	D3 OEO	D4 LPS	D5 LPS+GPA	D6 LPS+OEO	P Value
1	131.9 t	131.7 t	132.1 t	131.6 t	132.4 t	132.0 t	131.9 t	0.136
7	148.7 u	148.0 a, u	142.5 b, u	143.5 b, u	159.3 c, u	150.0 a, u	150.0 a, u	0.00043
14	158.8 v	155.2 a, v	151.5 b, v	152.4 b, v	170.5 c, v	161.0 d, v	162.0 d, v	0.00035
21	193.1 w	186.0 a, w	182.5 b, w	183.0 b, w	209.0 c, w	197.0 d, w	201.0 e, w	0.00032
28	171.5 x	161.0 a, x	160.5 b, x	161.0 b, x	189.5 c, x	178.0 d, x	179.0 e, x	0.00062
35	155.7 y	154.5 a, v	150.5 b, v	151.0 b, v	162.5 c, y	157.0 a, y	158.6 d, y	0.00056
42	144.0 z	144.5 a, y	136.5 b, y	140.0 c, y	151.5 d, z	145.5 a, z	146.0 a, z	0.00082
	means	154.4 ac	150.8 bc	151.8 c	167.2 d	160.0 e	161.2 e	0.00085

a, b, c, d, e Within the same row, means with the same letters are not statistically different (P<0.05). t, u, w, x, y, z Within the same column, means with the same letters are not statistically different (P<0.05).

reduced the concentration of triglycerides in the blood when compared to the basal diet, an effect that is also reflected in the diets that only had LPS and those that had LPS+GPA. GPA and OEO positively influenced intestinal inflammation; however, they did not present statistically significant differences between them (D5 and D6).

Table 6. Triglycerides (mg dL^{-1}) in blood serum of broilers fed with the addition of oregano essential oil (*Lippia origanoides*) in an *in vivo* intestinal inflammation model.

Day	Means	D1 control	D2 GPA	D3 OEO	D4 LPS	D5 LPS+GPA	D6 LPS+OEO	P value
1	128.1 t	128.1 t	127.8 t	128.4 t	127.9 t	128.0 t	128.3 t	0.092
7	164.3 u	161.0 a, u	150.5 b, u	153.5 b, u	177.2 c, u	170.7 d, u	173 d, u	0.00085
14	181.4 v	171.6 a, v	163.0 b, v, x	166.0 b, v	204.1 c, v	192.2 d, v	191.2 d, v	0.00092
21	203.1 w	199.3 a, w	194.1 b, w	195.5 b, w	219.3 c, w	204.5 d, w	205.0 d, w	0.00079
28	184.8 x	171.5 a, v	166.3 b, v	167.0 b, v	212.7 c, x	195.0 d, v	196.5 d, x	0.00066
35	173.2 y	166.5 a, x	161.0 b, x	162.0 b, x	200.2 c, y	187.0 d, x	189.7 d, v	0.00072
42	153.8 z	141.5 a, y	134.0 b, y	133.5 b, y	176.5 c, u	167.5 d, u	169.5 d, y	0.00086
	means	162.8 a	156.7 b	158.1 b	186.4 c	177.9 d	179.0 d	0.00091

a, b, c, d, e within the same row, means with the same letters are not statistically different (P<0.05). t, u, w, x, y, z within the same column, means with the same letters are not statistically different (P<0.05).

Creatinine. Mean creatinine values ranging from 0.235 mg dL^{-1} (day 1) to 0.373 mg dL^{-1} (day 21) had a statistically significant difference between all ages (P<0.05), except between day 7 and day 42 (P>0.05). In the different diets, averages from 0.278 mg dL^{-1} (D2) and 0.355 mg dL^{-1} (D4) were found. There was no statistically significant difference (P>0.05) between D2 and D3 (lowest values), but there was a significant difference (P<0.05) between D1 and D4 (Table 7). Besides, no significant statistical differences were found between D1, D5, and D6 (P>0.05). Creatinine is produced endogenously from creatine and creatine phosphate as a result of muscular metabolic processes and it is eliminated through the kidney by glomerular filtration. The determination of serum creatinine is useful for the diagnosis and control of acute and chronic kidney

diseases, and the estimation of glomerular filtration (Perazzi and Angerosa, 2011). Some studies have reported creatinine values between 0.44 and 1.24 mg dL^{-1} (Bueno *et al*., 2017), and others between 0.17 and 0.20 mg dL^{-1} (Broch *et al*., 2017). OEO affected the serum creatinine reduction in comparison with that of GPA. The use of LPS in an *in vivo* intestinal inflammation model showed to affect the serum creatinine, increasing its levels. The use of GPA and OEO significantly mitigated the creatinine rise generated by LPS.

Table 7. Creatinine (mg dL^{-1}) in blood serum of broilers fed with the addition of oregano essential oil (*Lippia origanoides*) in an *in vivo* intestinal inflammation model.

Day	Means	D1 control	D2 GPA	D3 OEO	D4 LPS	D5 LPS+GPA	D6 LPS+OEO	P value
1	0.235 t	0.234 t	0.235 t	0.237 t	0.232 t	0.236 t	0.235 t	0.247
7	0.281 u	0.279 a, u	0.260 b, u	0.265 b, u	0.310 c, u	0.285 a, uy	0.285 a, u	0.022
14	0.303 v	0.303 a, v	0.285 b, v	0.290 b, v	0.340 c, v	0.303 a, vy	0.305 a, v	0.035
21	0.373 w	0.373 a, w	0.325 b, w	0.345 c, w	0.440 d, w	0.375 a, w	0.380 a, w	0.015
28	0.340 x	0.335 a, x	0.305 b, x	0.315 b, x	0.390 c, x	0.340 a, x	0.345 a, x	0.017
35	0.297 v	0.297 a, y	0.278 b, v	0.280 b, v	0.332 c, y	0.295 a, y	0.301 a, v	0.021
42	0.272 u	0.274 a, u	0.255 b, u	0.254 b, u	0.295 c, z	0.276 a, u	0.275 a, u	0.031
	means	0.298 a	0.278 b	0.285 b	0.335 c	0.301 a	0.304 a	0.028

a, b, c, d, e within the same row, means with the same letters are not statistically different (P<0.05). t, u, w, x, y, z within the same column, means with the same letters are not statistically different (P<0.05).

Phosphorous (P). For the variable phosphorus, average values from 7.100 mg dL^{-1} (Day 1) to 9.022 mg dL^{-1} (Day 42) were found with a statistically significant difference between the different evaluation ages (P<0.05). In the different diets evaluated, average values from 7.304 mg dL^{-1} (D4) to 8.662 mg dL^{-1} (D2) were registered with statistically significant differences (P<0.05) between all treatments (P>0.05), except for D2 and D3 (highest values) (Table 8). P is one of the macrominerals with the highest concentration in organisms; it is considered an essential mineral for the metabolism of the animal organism, playing a vital role in the development and maintenance of bone structures, an aspect of great importance in poultry farming. When animals have highly accelerated muscle development, bone development must be consistent with it. P is also a component of ATP and nucleic acids and is part of the phospholipids that comprise and give flexibility to cell membranes (Díaz-González *et al.,* 2012). Some studies have reported values between 2.5 and 3.1 mg dL^{-1} (Karimzadeh *et al.,* 2017), and 6.50 and 7.63 mg dL^{-1} (Díaz-López *et al*., 2014). The results of these studies show an increase in blood P levels in animals fed the diets containing growth promoters; conversely, a reduction of P was also evidenced in animals subjected to LPS and an evident improvement in this response with the addition of the two promoters (GPA and OEO).

Calcium. Mean blood calcium values ranged from 8.89 mg dL^{-1} (Day 1) to 11.49 mg dL^{-1} (Day 42), with statistically significant differences for all evaluation ages (P<0.05). The diets 9.798 mg dL^{-1} (D4) and 10.74 mg dL^{-1} (D2) had a statistically significant difference between all diets (P<0.05), except (P>0.05) between D2 and D3, and between D5 and D6 (Table 9). Calcium is one of the macrominerals that are in the highest concentration in the animal organism; it is vital for the maintenance of muscle function and bone structure, and necessary for intestinal microorganism functions. The calcium concentrations reported in the current study are similar to other investigations reporting values between 9.72 and 11.10 mg dL^{-1} (Díaz-González *et al.,* 2012; Karimzadeh *et al*., 2017). The results show the positive effect that growth promoters (D2 and D3) have when compared to the basal diet (D1). The effect of reducing blood calcium in animals that consumed the diet with LPS is also evidenced, in addition to the positive recovery effect against the LPS exposure/challenge shown by those that consumed GPA (D5) and OEO (D6).

Table 8. Phosphorus (mg dL^{-1}) in blood serum of broilers fed with the addition of oregano essential oil (*Lippia origanoides*) in an *in vivo* intestinal inflammation model.

Day	Means	D1 control	D2 GPA	D3 OEO	D4 LPS	D5 LPS+GPA	D6 LPS+OEO	P value
1	7.112 t	7.115 t	7.100 t	7.120 t	7.108 t	7.112 t	7.118 t	0.347
7	7.330 u	7.715 a, u	7.955 b, u	7.980 b, u	6.400 c, u	7.290 d, u	6.640 e, u	0.0042
14	7.675 v	8.012 a, v	8.495 b, v	8.370 b, v	6.640 c, v	7.573 d, v	6.960 e, v	0.0057
21	8.040 w	8.160 a, v	8.850 b, w	8.805 b, w	7.220 c, w	7.680 d, v	7.525 d, w	0.0068
28	8.287 x	8.575 a, w	9.130 b, x	9.065 b, x	7.247 c, w	7.930 d, w	7.776 d, x	0.0043
35	8.761 y	8.630 a, w	9.475 b, y	9.425 b, y	8.080 c, x	8.510 d, x	8.450 d, y	0.0081
42	9.022 z	8.945 a, x	9.631 b, y	9.536 b, y	8.445 c, y	8.865 d, y	8.715 d, z	0.0062
	means	8.162 a	8.662 b	8.611 b	7.304 c	7.849 d	7.595 e	0.0060

a, b, c, d, e within the same row, means with the same letters are not statistically different (P<0.05). t, u, w, x, y, z within the same column, means with the same letters are not statistically different (P<0.05).

Table 9. Calcium (mg dL^{-1}) in blood serum of broilers fed with the addition of oregano essential oil (*Lippia origanoides*) in an *in vivo* intestinal inflammation model.

Day	Means	D1 control	D2 GPA	D3 OEO	D4 LPS	D5 LPS+GPA	D6 LPS+OEO	P value
1	8.879 t	8.870 t	8.890 t	8.875 t	8.884 t	8.870 t	8.890 t	0.561
7	9.635 u	9.780 a, u	10.04 b, u	9.980 b, u	9.135 c, u	9.440 d, u	9.435 d, u	0.011
14	10.15 v	10.15 a, v	10.50 b, v	10.49 b, v	9.470 c, v	10.15 a, v	10.13 a, v	0.009
21	10.38 w	10.52 a, w	10.74 b, w	10.67 b, w	9.770 c, w	10.36 d, w	10.26 d, w	0.012
28	10.90 x	10.97 a, x	11.64 b, x	11.57 b, x	9.935 c, x	10.66 d, x	10.63 d, x	0.021
35	11.07 y	11.20 a, y	11.66 b, x	11.55 b, x	10.27 c, y	10.90 d, y	10.85 d, y	0.014
42	11.49 z	11.51 a, z	11.74 b, x	11.70 b, y	11.11 c, z	11.46 a, z	11.42 a, z	0.020
	means	10.43 a	10.74 b	10.69 b	9.798 c	10.26 d	10.23 d	0.011

a, b, c, d, e within the same row, means with the same letters are not statistically different (P<0.05). t, u, w, x, y, z within the same column, means with the same letters are not statistically different (P<0.05).

CONCLUSIONS

The addition of GPA in the diet had a positive effect when compared to the basal diet. Moreover, the addition of OEO in the diet improved productive and metabolic indicators, equaling, and in some cases, exceeding the results obtained using GPA. The addition of LPS as an *in vivo* inflammation model had a negative effect on productive and metabolic variables. On the contrary, the addition of OEO showed a positive effect on zootechnical parameters and blood metabolites of broilers subjected to an *in vivo* intestinal inflammation model with the addition of *E. coli* LPS. Therefore, OEO could be considered to partially or totally replace the use of GPA.

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Effectiveness of postharvest calcium salts applications to improve shelf-life and maintain apricot fruit quality during storage

Efectividad de las aplicaciones de sales de calcio poscosecha para mejorar la vida útil y mantener la calidad de la fruta de albaricoque durante el almacenamiento

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ABSTRACT

Fresh apricot is a nutritious and popular fruit because of its special aroma and taste. However, high ripening rates and susceptibility to mechanical injury and postharvest diseases limit its shelf life. Therefore, the effect of immersion in different calcium salts solution on the qualitative and biochemical characteristics of fresh apricot fruit 'Shahroudi' cv was evaluated. Treatments were solutions of calcium chloride ($CaCl_2$), calcium nitrate $Ca(NO_3)_2$, and calcium sulfate ($CaSO_4$) at a concentration of 1 or 2%. Distilled water was used as a control. Fruits were immersed in water or calcium salts solution for 2 min, air-dried, and then stored at 2±1 °C for three weeks. However, the highest firmness was related to 2% $CaCl_2$ treatment, which was about 60% higher than control, followed by 1% $Ca(NO_3)_2$ treatment (50% more than control). Also, the lowest Total Soluble Solids value was 10.46%, which was obtained from 2% $CaCl_2$ and the highest value was obtained in control (15.1%). Besides, the mentioned treatments improved the nutritional value of apricot fruit by increasing total phenolic compounds, and tissue calcium content. The shelf life was 15.67 days in control, while 2% $CaCl_2$ treatment doubled (35.33 days) the shelf life of apricot fruit. In general, the best result was obtained using $CaCl_2$ at 2% treatment for 2 min, which may be applied as a postharvest treatment to improve the storage life of apricot fruit 'Shahroudi' cv.

Keywords:
Calcium
Phenol
Postharvest
Shelf life
Storage

RESUMEN

El albaricoque fresco es una fruta nutritiva y popular debido a su aroma y sabor especial. Sin embargo, las altas tasas de maduración y la susceptibilidad a daños mecánicos y enfermedades de poscosecha limitan su vida útil. Por tanto, se evaluó el efecto de la inmersión de la fruta en diferentes soluciones de sales de calcio sobre las características cualitativas y bioquímicas del fruto fresco de albaricoque cv. 'Shahroudi'. Los tratamientos incluyeron control (agua destilada) y soluciones de cloruro de calcio ($CaCl_2$), nitrato de calcio $Ca(NO_3)_2$ y sulfato de calcio ($CaSO_4$) en una concentración de 1 o 2%. Las frutas se sumergieron en agua o en una de las soluciones de sales de calcio durante 2 min, se secaron al aire y luego se almacenaron a 2±1 °C durante tres semanas. Sin embargo, la mayor firmeza se relacionó con el tratamiento con $CaCl_2$ al 2%, que fue aproximadamente un 60% más alto que el control, seguido del tratamiento con $Ca(NO_3)_2$ al 1% (50 % más que el control). obtenido a partir de $CaCl_2$ al 2% y el mayor valor se obtuvo en el control (15,1%). Además, los tratamientos mencionados mejoraron el valor nutricional de los frutos de albaricoque al aumentar los compuestos fenólicos totales y el contenido de calcio en los tejidos. La vida útil fue de 15,67 días para el control, mientras que, el tratamiento con $CaCl_2$ al 2% duplicó (35,33 días) la vida útil de la fruta de albaricoque. En general, el mejor resultado se obtuvo usando el tratamiento 2% de $CaCl_2$ durante 2 min, el cual se podría aplicar como tratamiento de poscosecha para mejorar la vida de almacenamiento de la fruta de albaricoque cv. 'Shahroudi'.

Palabras clave:
Calcio
Fenol
Poscosecha
Vida útil
Almacenamiento

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Qualitative and quantitative losses of fresh products occur in all stages (from harvest to final delivery to the consumer) (Benichou *et al*., 2018). To reduce these losses, producers and operators must prioritize the biological and environmental factors involved in degradation and adopt postharvest techniques that delay aging and maintain the best possible quality. Apricots have a short postharvest life and are very sensitive to damage due to their soft and juicy texture. This fruit has a high nutritional value, but due to the rapid softening of the fruit texture, it has a short shelf life and loses its quality in a short time (Moradinezhad and Dorostkar, 2020). The increased demand for fruits in the international markets implies continuous research and improvement of technologies to maintain quality and extend the shelf life of produce (Benichou *et al*., 2018).

It is well known that calcium plays a major role in maintaining the quality of fruit and vegetables (Singh *et al*., 2021; Oliveira *et al*., 2016; Boshadi *et al.,* 2018). The immersion method can be more useful than spray to apply postharvest treatments (Ghasemi *et al*., 2021). Postharvest calcium has been demonstrated to maintain membrane integrity and firmness of fruit tissue, which increases the shelf life of fresh produce (Moradinezhad *et al*., 2019; Li *et al*., 2020). Manganaris *et al*. (2007) examined the postharvest effect of calcium salts on peach fruit. They showed that pulp calcium increased by 74% and reduced the severity of cold injury symptoms, ethylene levels, pectin polygalacturonase, and pectin methylesterase. Another study showed that the postharvest application of 3% calcium chloride on apricot fruit not only maintained sensory properties but also increased the strength and durability of the cell wall and also significantly reduced the microbial load (Sartaj *et al*., 2013).

Shahroudi apricot cultivar is one of the important commercial cultivars of Iran. However, there is scarce information about the effect of postharvest calcium salts solution dipping on the physicochemical properties of apricot fruit cultivar 'Shahroudi'. Therefore, this study aimed to investigate the effect of postharvest application of different calcium salts (calcium chloride, calcium nitrate, and calcium sulfate) on the quality of 'Shahroudi' apricot fruit.

MATERIALS AND METHODS

Plant material

Apricots of the cultivar Shahroudi were harvested in the east region of Iran at the commercial maturity stage (total soluble solids about 11% and firmness 20N). This research was conducted late in May 2018. The fruits were then transferred to the postharvest laboratory, Faculty of Agriculture, University of Birjand. 200 fruits (weighing about 55 g) were considered for this experiment. After sorting, the fruits were washed with distilled water to remove any dust.

Treatments

Apricot fruits were divided into seven groups and then dipped in water or solutions of calcium salts at 25 °C for 2 min as follows:

[1] Distilled water (control).
[2] Calcium chloride solution 1% ($CaCl_2$ 1%).
[3] Calcium chloride solution 2% ($CaCl_2$ 2%).
[4] Calcium nitrate solution 1% ($Ca(NO_3)_2$ 1%).
[5] Calcium nitrate solution 2% ($Ca(NO_3)_2$ 2%).
[6] Calcium sulfate solution 1% ($CaSO_4$ 1%).
[7] Calcium sulfate solution 2% ($CaSO_4$ 2%).

All treated fruits were allowed to air dry at room temperature (25 °C) for 1 h. Thereafter, the apricots were placed in polyethylene containers (dimension: 9×9×12 cm) with 500 mL volume and wrapped with double-layer cellophane, and then stored in a cool room at 2±1 °C with 85±5% relative humidity for three weeks. Important parameters such as temperature and relative humidity that directly affect the results were controlled during the experiment. All traits (except shelf life) were measured and evaluated after 3 weeks.

Firmness, total soluble solids (TSS), and titratable acidity (TA)

To measure the firmness of fruits, a digital penetrometer (Fruit Hardness Tester, Model FHT 200, Extech Co., USA) and a 2 mm probe were used. Data were presented in Newton units. Total soluble solids in fruit juice were measured using a hand-held refractometer (RF 10, Brix 0-32%, Extech Co., USA), and data were expressed as percentages (%). The titration method was used to evaluate the titratable acidity expressed as the percentage of malic acid.

Total phenolic content (TPC), calcium content, and fruit shelf life

Total phenolic content was determined using the Folin-Ciocalteau method. The absorbance at 755 nm was measured using a spectrophotometer (Bio Quest, CE 2502). The final results were expressed as mg of Gallic acid (EAG) 100 g^{-1} of fresh apricot. The calcium content of fruit tissue was determined using the acid digestion method. The calcium ion level was measured with an atomic absorption spectrophotometer, and the results were expressed as mg Ca 100 g^{-1} dry weight (DW).

Fruit shelf life was assessed using visual observations and acceptability for presentation to the consumer. This parameter was expressed in terms of days (Moradinezhad and Jahanani, 2016).

Experimental design and statistical analysis

This experiment was carried out in a completely randomized design with seven treatments and four replications. Statistical analysis was performed with GenStat (Discovery Edition, version 12.1, 2009, VSN, International, UK) and Excel. Also, the LSD test at the level of 1% (P<0.01) was used to identify significant differences between means. In the present study, mean ± standard error was used to present the data better.

RESULTS AND DISCUSSION

Firmness

Dipping apricots in different calcium salts had a significantly affected on maintaining tissue firmness compared to control samples (Figure 1). At the end of the storage period, it was found fruits treated with $CaCl_2$ at 2% had the highest (18.1N) firmness while control fruit exhibited the lowest (10.6N) firmness. However, firmness was 20 N on the first day (see materials and methods) and these results showed that 2% $CaCl_2$ treatment had the least change in tissue firmness compared to the day of harvest. On the other hand, evaluations showed that by increasing the concentration of all used calcium salts from 1% to 2%, the firmness of apricot fruit tissue was significantly maintained.

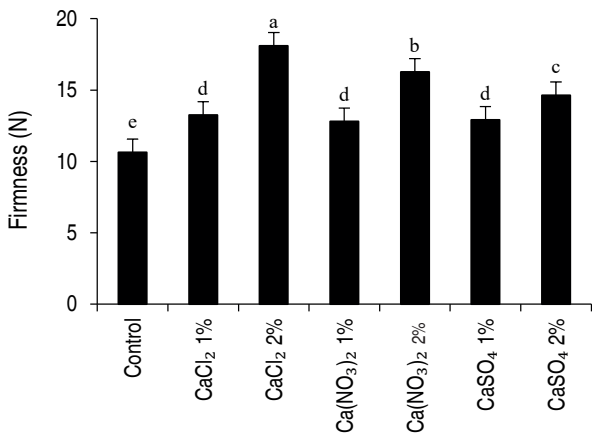

Figure 1. Effect of dipping in different calcium salts solution on the firmness of apricot fruit stored at 2±1 ˚C for 21 days. $CaCl_2$ (calcium chloride), $Ca(NO_3)_2$ (calcium nitrate), $CaSO_4$ (calcium sulfate).Error bars represent the error deviation. Symbols with the same letter are not significantly different between them at P<0.01 (LSD test).

It has been proven that ethylene production in fruit tissue (climacteric fruits) causes the fruit to ripen and soften. In the report on apple fruit, Mohebbi *et al*. (2020) showed that postharvest application of calcium chloride reduced ethylene production and subsequently increased the firmness of fruit tissue; they stated that lower ethylene production in Ca-treated fruit may be due to its contribution to the maintenance of cell membranes, delaying ACO-catalyzed conversion of ACC into ethylene. An important function of Ca in plants is to increase the rigidity of the cell wall and to promote the cohesion of neighboring cells. This positive effect of calcium on firmness has been widely reported by other researchers (Ortiz *et al*., 2011; Moradinezhad and Jahanani, 2016).

On the other hand, the results showed that among different calcium salts, calcium chloride (2%) maintained fruit tissue firmness more than calcium nitrate, and calcium sulfate.

This effect is probably due to the formation of calcium pectate through the reactions of calcium with pectic acid, calcium chloride increases the molecular bond between cell wall components more than other calcium salts and strengthens the pectin chain. This scenario was consistent with the results of Manganaris *et al.* (2007) using peaches.

Total soluble solids (TSS)

According to Table 1, calcium salts treatment had significant effects on the TSS. In all treatments, TSS was lower compared to control fruits, and the lowest TSS was obtained from the 2% $CaCl_2$ treatment.

During storage and ripening, starch present in the fruits converts slowly and gradually into sugar. Sajid *et al.* (2019) reported that calcium changes the function of enzymes such as pectinase, methylesterase, and polygalacturonase. It seems that calcium treatment can delay ripening, senescence, and respiration rate, which reduces the amount of total soluble solids. Since calcium slows down respiration and metabolism and also slows down the hydrolysis of polysaccharides to monosaccharides, it leads to the delay of ripening and reduces the TSS of fruit during storage (Ranjbar *et al.*, 2018).

Table 1. Effect of dipping in different calcium salts solution on total soluble solids (TSS), titratable acidity (TA), total phenolic content (TPC), and calcium content of tissue of apricot fruit stored fruit at 2±1 °C for 21 days.

Treatment		TSS (%)	TA (%)	TPC (mg EAG 100 g^{-1} FW)	Ca (mg 100 g^{-1} DW)
Control		15.1±0.3 a*	0.75±0.0 d	62.94±0.0 e	49.24±0.6 d
$CaCl_2$	1%	11.9±0.1 c	1.56±0.1 b	63.55±0.0 c	89.37±1.2 a
$CaCl_2$	2%	10.4±0.0 d	1.96±0.0 a	63.93±0.1 a	90.59±1.4 a
$Ca(NO_3)_2$	1%	11.5±0.0 c	1.70±0.0 b	63.74±0.0 b	57.56±0.9 bc
$Ca(NO_3)_2$	2%	11.8±0.0 c	1.88±0.0 a	63.91±0.0 a	59.68±1.1 b
$CaSO_4$	1%	13.0±0.0 b	1.40±0.0 c	63.13±0.1 d	57.05±0.1 bc
$CaSO_4$	2%	12.9±0.1 b	1.25±0.1 cd	63.61±0.1 c	58.43±0.5 b
Level of Sign (1%)		**	**	**	**
LSD		1.29	0.18	0.18	6.97

*Means±SE followed by different letters in the same column for the same evaluated parameter are significantly different ($P \leq 0.01$) according to the LSD test. $CaCl_2$ (calcium chloride), $Ca(NO_3)_2$ (calcium nitrate), $CaSO_4$ (calcium sulfate). FW: Fresh weight, and DW: Dry weight.

Titratable acidity (TA)

After 21 days of storage, the TA in all treated fruit increased compared to the control samples. The results showed that the postharvest application of calcium salts had a significant effect on the TA of apricot fruit. The highest TA value was obtained from $CaCl_2$ 2% (1.96%) and $Ca(NO_3)_2$ (1.88%) treatments and, the lowest value was observed in control samples (0.75%) (Table 1).

The titratable acidity is directly related to the concentration of organic acids present in the fruit, which are an important parameter in maintaining the quality of fruits. In apricot, malic acid is the principal acid. It has been proved that acidity decreases during ripening due to the breakup of acid into sugars. Liu *et al.* (2017) reported that calcium chloride maintains the titratable acidity of the fruit; they stated that calcium treatment could reduce acid oxidation. Similarly, Ranjbar *et al.* (2018) on apple and Ishaq *et al.* (2009) on apricot fruit reported that the calcium chloride application maintained acidity during storage.

Total phenolic compounds (TPC)

As shown in Table 1, all postharvest calcium salts dips had a significant effect on the TPC of apricot fruit. The highest TPC values were obtained using $CaCl_2$ and $Ca(NO_3)_2$ treatments at 2%.

Phenolic compounds in fruits and vegetables can protect cells against oxidative injury through scavenging free

radicals. Jacobo-Velázquez *et al*. (2011) suggested a model or the mechanism of action of calcium on phenol content. They stated that cytosolic ATP is released in stressed and damaged cells. This ATP is received by membrane receptors. This process signals and produces activated oxygen, which in turn increases the rate of mitochondrial respiration. Increased reactive oxygen activates phenol synthesis pathways. Accumulation of phenolic molecules in plant tissues is done to neutralize and scavenger free radicals (antioxidants). Also, Ranjbar *et al*. (2018) reported that calcium increases the amount of total phenol in apple fruit; since calcium rises the polysaccharide content in the fruit cell wall and the permeability of the membrane, therefore, the wall strength, and cell membrane can be maintained, which lead to preventing the oxidation of phenolic compounds. Similar to the present study, $CaCl_2$ (2%) treatment significantly enhanced total phenols content in apricot fruits, suggesting that $CaCl_2$ (2%) and $Ca(NO_3)_2$ (2%) might be an efficient strategy to improve total phenols content in fruits such as apricot, which increases the nutritional value of this fruit.

Calcium content of fruit

As expected, all calcium salts treatments increased the calcium content of apricot fruit compared to the control. The highest value of calcium was obtained in $CaCl_2$ (1 or 2%) treatment. Also, in other treatments, the calcium content of fruit increased with an increment in concentration from 1 to 2% of applied salts (Table 1).

It has been proved that calcium is an essential substance in maintaining the structure of the cell wall, also; it is well known that it reduces ethylene and delays the ripening process of products (Mohebbi *et al*., 2020). Calcium treatment is one of the safe treatments that, in addition to maintaining the quality of the fruit, it is also non-toxic. Similarly, Madani *et al*. (2016) reported that using calcium (spray) on papaya fruits significantly increased the calcium content of the pulp and peel of the fruit. They exposed a marked increase in calcium content in papaya fruit suggesting that exogenous calcium might be able to cross the fruit epidermis and incorporate it into the cell wall matrix of fruit cells.

Shelf life

The shelf life of apricot fruits was evaluated during cold storage. As shown in Figure 2, the shelf life in all calcium treatments was extended compared to the control. However, the highest shelf life was obtained using $CaCl_2$ at 2% treatment (35.33 days), while the shelf life of the control samples was only 15.67 days.

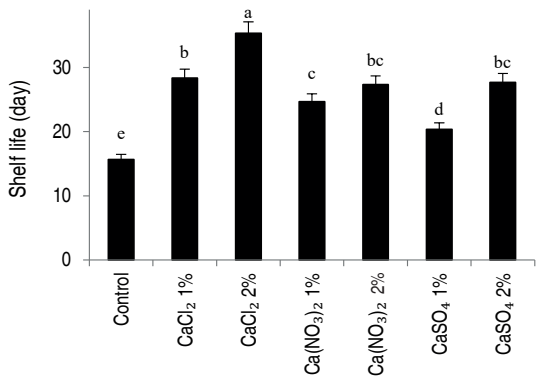

Figure 2. Effect of dipping in different calcium salts solution on the shelf life of apricot fruit stored at 2±1 °C for 21 days. $CaCl_2$ (calcium chloride), $Ca(NO_3)_2$ (calcium nitrate), $CaSO_4$ (calcium sulfate). Error bars represent the error deviation. Same letter are not significantly different between them, at $P<0.01$ (LSD test).

Fruit shelf life is dependent mainly on firmness and cell wall composition. During apricot postharvest storage, firmness gradually decreases while pectin is gradually depolymerized and degraded (Gao *et al*., 2020). Liu *et al*. (2017) reported that apricot treatment with 1% calcium chloride followed by cold storage at 5 °C extends shelf life, maintains a firmer texture, and reduces the degradation of cell wall polysaccharide main and side chains in apricot fruit. The results of this study showed that treatment with three forms of calcium salts had a

positive effect on the postharvest quality and prolonged shelf life of apricots. Also, the shelf life results agreed well with those from tissue firmness and decay, suggesting that the short shelf life was related to tissue cell wall endurance. Previously, Moradinezhad and Jahani (2016) have also described similar views that calcium application delays extended shelf life and retards decomposition during cold storage in apricot fruit.

CONCLUSION

In conclusion, we found the positive effect of calcium chloride ($CaCl_2$) and calcium nitrate ($Ca(NO_3)_2$) postharvest application on enhancing the total phenols of apricot fruit 'Shahroudi' cv. was accompanied by increased firmness, the calcium content of pulp, and titratable acidity. Also, results showed that the application of the mentioned calcium salts (2% $CaCl_2$ and 1% $Ca(NO_3)_2$) significantly extended the apricot fruit shelf life. Therefore, these treatments may be recommendable for practical use in this kind of fruit.

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Review article

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Revista Facultad Nacional de Agronomía

Antimicrobial potential of camu camu (*Myrciaria dubia*) against bacteria, yeasts, and parasitic protozoa: a review

Potencial antimicrobiano del camu camu (*Myrciaria dubia*) contra bacterias, levaduras y protozoos parásitos: una revisión

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ABSTRACT

Some microorganisms are responsible for food spoilage and foodborne infections worldwide. These microorganisms are becoming increasingly resistant to degradation or inhibition due to exposure to antibiotics, antifungal, and antiparasitics, posing a growing threat to human health. The aim of this study was to describe the antimicrobial properties of compounds present in *Myrciaria dubia* (pulp, seed, peel, and leaves) against bacteria (*Staphylococcus spp.*, *Escherichia coli*, *Salmonella* and others), yeasts (*Candida albicans* and *Saccharomyces cerevisiae*) and parasitic protozoa (*Leishmania amazonensis* and *Plasmodium falciparum*). Different papers published in the main databases (Scopus, ScienceDirect, PubMed, Wiley Online Library, as well as in university repositories) were reviewed. These results were analyzed and organized according to their inhibitory activity, attributable metabolic actions of this plant, mainly based on its phenolic compounds present (rhodomyrtone, isomyrtucommulone B, myrciarone B, trans-resveratrol, 2.4-dihydroxybenzoic acid, myricetin, syringic, ellagic acid and casuarictin), which can inhibit the synthesis or destabilize the microbial membrane, nucleic acids, cell walls in bacteria and mitochondrial dysfunction in protozoa.

Keywords:
Ascorbic acid
Inhibition
Pathogen
Phenolic compounds

RESUMEN

Algunos microorganismos son responsables del deterioro de los alimentos y de las infecciones alimentarias en el mundo. Estos microorganismos se están volviendo cada vez más resistentes a la degradación o inhibición debido a la exposición de antibióticos, antifúngicos y antiparasitarios, lo que supone una amenaza creciente para la salud de las personas. El objetivo de este estudio fue describir las propiedades antimicrobianas de los compuestos presentes en *Myrciaria dubia* (pulpa, semilla, cáscara y hoja) contra bacterias (*Staphylococcus* spp., *Escherichia coli*, *Salmonella* y otros), levaduras (*Candida albicans* y *Saccharomyces cerevisiae*) y protozoos parásitos (*Leishmania amazonensis* y *Plasmodium falciparum*). Se revisaron distintos trabajos publicados en las principales bases de datos (Scopus, ScienceDirect, PubMed, Wiley Online Library), así como en repositorios de universidades. Estos resultados fueron analizados y organizados de acuerdo a su actividad inhibitoria, capacidad atribuible a las acciones metabólicas de esta planta, basados principalmente en sus compuestos fenólicos presentes (rhodomyrtone, isomyrtucommulone B, myrciarone B, trans-resveratrol, ácido 2,4-dihidroxibenzoico, myricetin , syringic, ácido elágico y casuarictin), los que pueden inhibir la síntesis o desestabilizar la membrana microbiana, ácidos nucleicos, paredes celulares en bacterias y disfunción mitocondrial en protozoos.

Palabras clave:
Ácido ascórbico
Inhibición
Patógenos
Compuestos fenólicos

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Infectious diseases are caused by pathogenic microorganisms such as bacteria, yeast, and parasitic protozoa (WHO, 2021). Their spread is increasing, producing economic crises and threatening people's safety (Gushulak and MacPherson, 2004; Vignier and Bouchaud, 2018). Over the years, microorganisms have acquired resistance to different antibiotics, antifungals, or antiparasitic; their mechanisms of resistance to different drugs have increased due to the non-specific rejection of hydrophobic chemical substances due to the impermeability of the outer membrane, the acquisition of non-specific eflux pumps, biofilms formation, and others. Thus, it is necessary to search for natural sources of antimicrobials agents that do not affect humans' and animals' health nor harm the environment (Carey and McNamara, 2015; Mir, 2022; Moglad *et al*., 2020; Samanta and Bandyopadhyay, 2020; Santos and Santana, 2019; Yadav *et al*., 2019).

Myrciaria dubia is a shrub belonging to the *Myrtaceae* family; native to the Amazon rainforest and grows naturally in floodable areas of streams and on the banks of rivers, lakes, or swamps of the Peruvian, Brazilian, Colombian, Ecuadorian, and Venezuelan Amazon (Castro *et al*., 2018; Hernández *et al*., 2011; Lim, 2012). The phytochemical properties of this plant have been the subject of multiple studies. They have been characterized and named as functional food, due to their high content of ascorbic acid, which varies according to the part of the fruit and its state of maturity (Alves *et al*., 2002; Castro *et al*., 2013; Cunha-Santos *et al*., 2019; Justi *et al*., 2000; Rodrigues *et al*., 2001; Santos *et al*., 2022; Villanueva-Tiburcio *et al*., 2010; Yuyama *et al*., 2002; Obregón-La Rosa *et al*., 2021).

M. dubia has been shown to contain carotenoids, such as β-carotene, violaxanthin, and luteoxanthin (Zanatta and Mercadante, 2007), saponins, tannins (Da Silva *et al*., 2022) and essential oils such as α-pinene, α-phellandrene, terpinolene, E-caryophyllene, γ-cadinene (Da Costa *et al*., 2022); in addition to phenolic compounds, proanthocyanidins (Fujita *et al*., 2013), quercetin and kaempferol derivatives (Gonçalves *et al*., 2010), delphinidin 3-glucoside, naringenin, cyanidin 3-glucoside, rutin, flavan-3-ol, flavonol, flavanone, and ellagic acid derivatives and catechin (Chirinos *et al*., 2010; Reynertson *et al*., 2008). Moreover, it presents phenolic compounds, such as vescalagin, castalagin (Fidelis *et al*., 2020); myrciarone A and B, isomyrtucommulone B, rhodomyrtone (Kaneshima *et al*., 2016), rosmarinic acid, trans-resveratrol, quercetin, syringic acid, methylvescalagin and cyanidin-3-glucoside, 2,4-dihydroxybenzoic acid (Do Carmo *et al*., 2019), myricetin and ellagic acid (De Azevêdo *et al*., 2014). The presence of these compounds varies according to the different parts of *M. dubia* (pulp, seed, peel, and leaves).

Some compounds present in *M. dubia* demonstrated antimicrobial activity (Figure 1) as myrciarone A from the peel (*Bacillus cereus, Bacillus subtilis, Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*), rhodomyrtone from the peel (*B. subtilis*, *B. cereus*, *M. luteus, S. aureus, S. epidermidis, Streptococcus mutans*), isomyrtucommulone B from the seed (*B. cereus, S. aureus, S. epidermidis*, *B. subtilis*) myrciarone B from the seed (*B. cereus*, *B. subtilis, S. aureus, S. mutans*, *S. epidermidis*) (Kaneshima *et al*., 2017), trans-resveratrol (*Schistosoma mansoni*), methylvescalagin (*S. mansoni, Plasmodium falciparum*), quercetin, 2,4-dihydroxybenzoic acid (*S. mansoni, P. falciparum* from the seeds (Do Carmo *et al*., 2020); myricetin, syringic acid, ellagic acid, and casuarictin from the lyophilized pulp powder proved to be effective against *S. aureus* (Fujita *et al*. 2015).

Some analyzes have shown that pure phenolic compounds such as quercetin, naringenin, and kaempferol have strong antimicrobial activity (Rauha *et al.,* 2000). Additionally, some compounds (Myricetin) present in *M. dubia* were found in other plant samples and demonstrated inhibitory action for *Proteus vulgaris* and *S. aureus* (Mori *et al*., 1987).

In this context, this review aimed to describe the antimicrobial properties of the compounds present in the pulp, seed, peel and leaves from *M. dubia* against bacteria, yeasts, and parasitic protozoa.

MATERIALS AND METHODS

From main databases, a search for papers published about this topic was performed (Scopus, ScienceDirect, PubMed, Wiley Online Library). Also, university repositories were consulted using the following descriptors *camu camu* or *Myrciaria dubia* and antimicrobial or bacteria or microorganisms or antimicrobial activity, preferably within the last 15 years. The information was organized according to the use of *M. dubia*, considering the antimicrobial properties of the compounds present

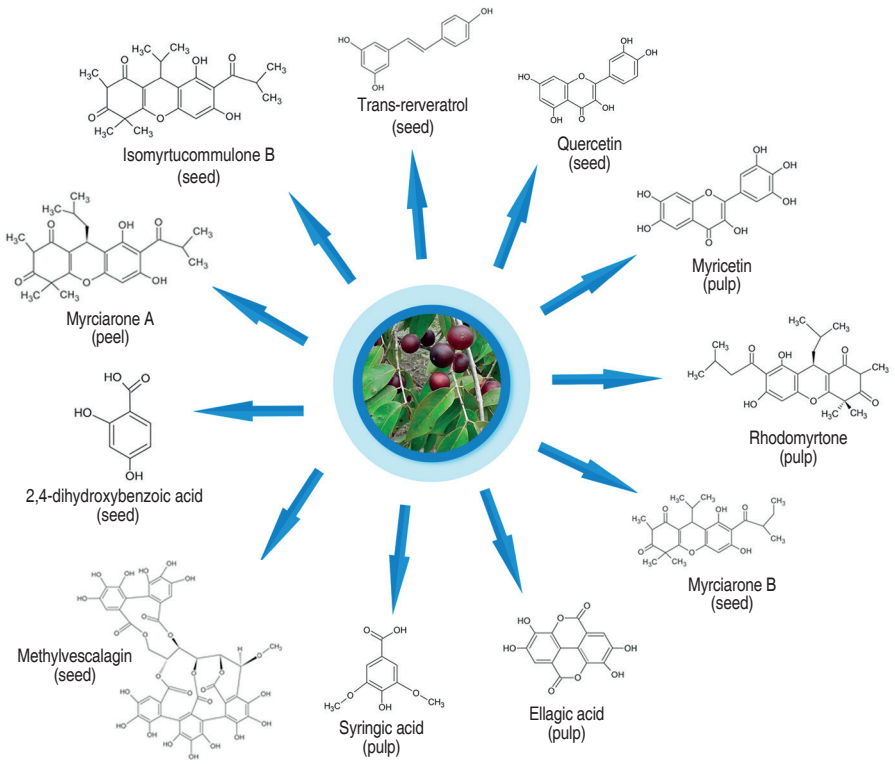

Figure 1. Phenolic compounds with antimicrobial properties from *M. dubia* (Kaneshima *et al*., 2017; Do Carmo *et al*., 2020; Fujita *et al.* 2015).

in the different parts of *M. dubia* against different microorganisms.

RESULTS AND DISCUSSION

Inhibitory capacity of *M. dubia*

M. dubia contains phenolic compounds (flavonoids and phenolic acids), and they can inhibit microorganisms (Kaneshima *et al*., 2017; Do Carmo *et al*., 2020; Fidelis *et al*., 2020).

The antimicrobial activity of phenolic compounds is related to the kinetic curve of microbial death and minimum inhibitory concentration (MIC) (Fujita *et al*., 2015; Levison, 2004; Pankey and Sabath, 2004).

Antibacterial activity is due to the compounds that degrade the cell wall and/or functionally interfere with the bacterial enzymes present in these structures (Finberg *et al*., 2004). They cause the death of microorganisms through a process known as bactericidal action. On the other hand, bacteriostatic action occurs when the ribosomal function and protein synthesis that allows the reduction of microbial population growth are inhibited (French, 2006).

Some compounds such as myricetin have shown a bacterial inhibition of RNA synthesis (*S. aureus*), this inhibitory action on DNA or RNA synthesis occurs due to the B ring of flavonoids, which interacts with hydrogen bonds causing stacking of nucleic acid bases (Mori *et al*., 1987). Likewise, Ohemeng *et al.* (1993) demonstrated that flavones (ellagic acid) inhibit the catalytic activity of DNA gyrase. Similarly, some alkaloids can act as agonists or antagonists of neuroreceptors/ion channels, leading parasites (*S. mansoni*) to death due to neurotoxic effects (Do Carmo *et al*., 2020).

Kaneshima *et al*. (2017) and Fidelis *et al*. (2020) demonstrated the antimicrobial activity of *M. dubia* on yeasts (*Candida albicans* and *Saccharomyces cerevisiae*). There is no knowledge about the mechanism of cellular action. Nevertheless, the inhibition of protozoa is possibly

due to the action of quercetin in causing mitochondrial dysfunction in these parasites (Correia *et al*., 2016).

***M. dubia* benefits.** The inhibitory capacity of *M. dubia* constitutes an excellent alternative as a functionalized ingredient in food; it can also be used in the pharmaceutical and cosmetic industries by presenting compounds with the biological activity of interest, in which ascorbic acid and phenolic compounds stand out (Conceição *et al*., 2020; Inocente-Camones *et al*., 2014; Fidelis *et al*., 2020).

The phenolic compounds present in *M. dubia* (pulp, seed, peel and leaves) have potential alternative uses, once they show inhibitory capacity against bacteria (*S. aureus, B. cereus, B. subtilis, S. mutans, S. epidermidis, E. coli, Streptococcus sanguinis*) and yeasts (*C. albicans*, *S. cerevisiae*) (Camere-Colarossi *et al*., 2016; Conceição *et al*., 2020; Fidelis *et al*., 2020; Fujita *et al*. 2015; Kaneshima *et al*., 2017; Myoda *et al*., 2010; Roumy *et al*., 2020). Additionally, the by-products can be used after a pre-treatment of drying with hot air, spray drying, or lyophilization (De Azevêdo *et al*., 2014; De Azevêdo *et al*., 2015). Furthermore, the lyophilized pulp of *M. dubia* has shown greater inhibition capacity than ampicillin (Fujita *et al*., 2013).

Another advantage of this plant is that contributes to human health as was demonstrated in different studies (Camere-Colarossi *et al*., 2016; De Azevêdo *et al*., 2014; Moromi *et al*., 2016; Myoda *et al*., 2010). It is traditionally used in the indigenous communities of Loreto, Peru to heal various illnesses, including gingivitis, and to keep the gums of human teeth healthy (Flores, 2010; Pinedo *et al*., 2011).

Inhibitory capacity of *M. dubia* against different microorganisms

The following is a summary of studies related to the inhibition of microorganisms (bacteria, yeasts and protozoa) for compounds present in the pulp, seed, peel, and leaves from *M. dubia*.

***M. dubia* against *Staphylococcus* spp.** The lyophilized optimized Camu-Camu seed extract (1g:20 mL of the mixture of 43.3% propanone, 40.7% water and 16% ethyl alcohol) showed inhibition against *S. aureus* ATCC13565 with an inhibition zone of 9.7 mm; it could block the transcription due to its castalagin and vescalagin contents (Fidelis *et al*., 2020). In another study, using n-hexane extract from *M. dubia* peel and seed; fractions of n-hexane extract (n-hexane layers and 90% acetonitrile layers) and acylphloroglucinols of n-hexane extract (1: Myrciarone A; 2: Rhodomyrtone; 3: Isomyrtucommulone B and 4: Myrciarone B) presented antimicrobial activity against *S. aureus*. In n-hexane extracted from the peel, MIC values were 12.50 (n-Hexane extract), 6.25 (n-hexane layers), 12.5 (90% acetonitrile layers), 1.56 (Myrciarone A) and 0.78 ug mL^{-1} (Rhodomyrtone). In n-hexane extracted from the seed obtained MIC value of 6.25 (n-Hexane extract, n-hexane layers, and 90% acetonitrile layers) and 1.56 ug mL^{-1} (Isomyrtucommulone B and Myrciarone B). Similarly, inhibitory activity was evidenced against *S. epidermidis* with MIC values of 6.25 (n-Hexane extract and n-hexane layers), 12.5 (90% acetonitrile layers), 3.13 (Myrciarone A) and 0.78 µg mL^{-1} (Rhodomyrtone) for the n-hexane extract from the peel and for the n-hexane extract from the seed were obtained MIC values of 12.5 (n-Hexane extract and n-hexane layers), 6.25 (90% acetonitrile layers and Isomyrtucommulone B) and 3.13 ug mL^{-1} (Myrciarone B), respectively (Kaneshima *et al*., 2015 and Kaneshima *et al*., 2017). Due to the presence of proanthocyanidins, anthocyanins, flavonoids, and phenolic acids in the lyophilized ethanol extract, *M. dubia* (aqueous extracts of seeds and peel) showed antimicrobial activity against *S. aureus* with an inhibition zone of 12 mm (De Azevêdo *et al*., 2014).

The methanolic extract obtained from *M. dubia* leaves (1.2 mg mL^{-1}) inhibited *S. epidermidis* 5001 by the action of β-sitosterol and betulinic acid, which allowed the activation of drug-like chemosensory signals (Roumy *et al*., 2020).

Additionally, antimicrobial activity of the methanolic extract (5 mg mL^{-1}) obtained from seed and peel of *M. dubia* for *S. aureus* was observed; the zone of inhibition for the seed extract was 2.7 mm while for the peel extract it was 3.1 mm. This is attributed to the high content of phenolic compounds (Myoda *et al*., 2010).

Another study showed that the antimicrobial activity of the lyophilized pulp extract of *M. dubia* diluted in 70% methanol inhibited *S. aureus* ATCC 29213 with a

MIC of 0.08 mg mL^{-1} (0% maltodextrin or gum arabic) presenting a higher activity than ampicillin (0.26 mg mL^{-1}), this antimicrobial activity is due to the presence of ellagic acid, tannins, cyanidin, quercetin, catechin, kaempferol, and rutin (Fujita *et al*., 2015).

The methanolic extracts obtained from seeds, peels, and leaves of *M. dubia* showed antimicrobial activity against *Staphylococcus* spp. as shown in Table 1. The extracts studied did not show inhibition against *S. aureus*. For *S. epidermidis* 8157, the inhibition occurred due to the action of β-sitosterol and betulinic acid present in the methanolic extract of leaves (Roumy *et al*., 2020).

Table 1. Antimicrobial effect of different parts of the fruit of *M. dubia*

Bacterium	MIC (mg mL^{-1})		
	Peel	Seed	Leaves
S. epidermidis 5001	0.30	0.30	
S. epidermidis 8157	0.15	0.15	1.20
S. epidermidis 10282	0.15	0.30	
S. lugdunensis T26A3	0.30	0.30	
S. warneri T12A12	0.30	0.30	

Another study carried out on lyophilized extracts obtained from 1 g of lyophilized *M. dubia* peel, pulp, and seeds and solvent ethanol and water (80/20, v/v) proved that the MIC for *E. coli* from the peel was 10 mg mL^{-1}. Extract with the most relevant antimicrobial potential was from pulp and seed parts (Conceição *et al*., 2020). This action was possible due to the formation of biofilms by their flavonoids myricetin and quercetin (Arita-Morioka *et al*., 2018).

***M. dubia* against yeasts.** The dry extract of *M. dubia* seed diluted with n-hexane (500 mg 10 mL^{-1}) showed inhibitory activity against *C. albicans* (MIC > 100 µg mL^{-1}); but the resulting layer of n-hexane and 90% acetonitrile layer obtained by countercurrent partitioning (acetonitrile: water = 9:1 v/v) and two isolated compounds such as isomyrtucommulone B and myrciarone B had no effect on the yeast (Kaneshima *et al*., 2017). In addition, Roumy *et al*., 2020 showed MIC values of 0.3, 0.3 and 1.2 mg mL^{-1} when they used diluted methanolic extracts (12 mg mL^{-1}) from peel, seed, and leaves respectively against *C. albicans* 10286. On the other hand, dry extracts from peel and seed diluted in water or DMSO to obtain concentrations of 0.1-5.0 mg mL^{-1} had no activity against *S. cerevisiae* (Myoda *et al*., 2010). In another study, an evaluation of the antimicrobial activity for *S. cerevisiae* NCYC1006 was carried out using the lyophilized optimized *M. dubia* seed extract (1:20 g:mL of the mixture of 43.3% propanone, 40.7% water, and 16% ethyl alcohol) presented an inhibition zone of 5.74 mm (Fidelis *et al*., 2020).

***M. dubia* against *Escherichia coli*.** The lyophilized optimized camu-camu seed extract (1:20 g:mL of the mixture of 43.3% propanone, 40.7% water, and 16% ethyl alcohol) presented antibacterial activity again *E. coli* IAL2064 with an inhibition zone of 6.64 mm. (Fidelis *et al*., 2020). This inhibition is probably caused by their phenolic compounds such as quercetin, catechin, gallic acid, ellagic acid, ellagitannins, and proanthocyanidins (Fujita *et al*., 2015). *M. dubia* fruit juice presented greater inhibitory capacity against *E. coli*, while for *Salmonella typhi*, its inhibition capacity was lower, with inhibition zones of 16.9 and 11.19 mm, respectively. This inhibition against *E. coli* and *S. typhi* is attributed to the low pH (2.09) of the fruit juice (López, 2017).

***M. dubia* against parasitic protozoa.** The action of dichloromethanolic extract from *M. dubia* leaves against *P. falciparum* (clone W2), *Leishmania amazonensis* (IFLA/BR/67PH8), *Leishmania braziliensis* (IOCL 566), and *Leishmania chagasi* (IOCL 579) through bioassays was evaluated. This extract showed inhibitory activity against *P. falciparum* (chloroquine-resistant strain W2). Also, it presented greater inhibitory activity against the *L. amazonensis* (200 µg mL^{-1} of extract inhibited in 85% of the promastigote form growth) than against *L. braziliensis* and *L. chagasi*. In addition, this extract presented a growth inhibition of 50% of the parasites (IC_{50}) equal to 2.35 µg mL^{-1} for *P. falciparum*,

190.73 µg mL^{-1} for *L. amazonensis,* and ≥ 200 µg mL^{-1} for *L. chagasi* and *L. braziliensis* (Correia *et al*., 2016).

Similarly, in an evaluation using different concentrations (10–500 µg mL^{-1}) from lyophilized extracts of *M. dubia* seeds (100/200 g:mL solvent (ultrapure water: ethanol)) with parasite suspensions (0.5% parasitaemia and 2% hematocrit), obtained IC_{50} of 24.2 µg mL^{-1} for *P. falciparum* (juvenile stage-12h) resistant to chloroquine (W2) with 100% H_2O and IC_{50} of 26.8 µg mL^{-1} for *P. falciparum* (trophozoite-24h) sensible to chloroquine (3D7) with 75% of H_2O + 25% ethanol extract, *in vitro* (Do Carmo *et al*., 2020), this may have occurred due to the action of phenolic compounds, flavonoids (quercetin) that allows the inhibition of enzymes (β-ketoacyl-ACP-reductase, β-hydroxacylACP-dehydratase and enoyl-ACP-reductase) involved in the type II fatty acid biosynthesis pathway (Tasdemir *et al*., 2006).

Some microorganisms which were inhibited by the action of *M. dubia* can be seen in Figure 2.

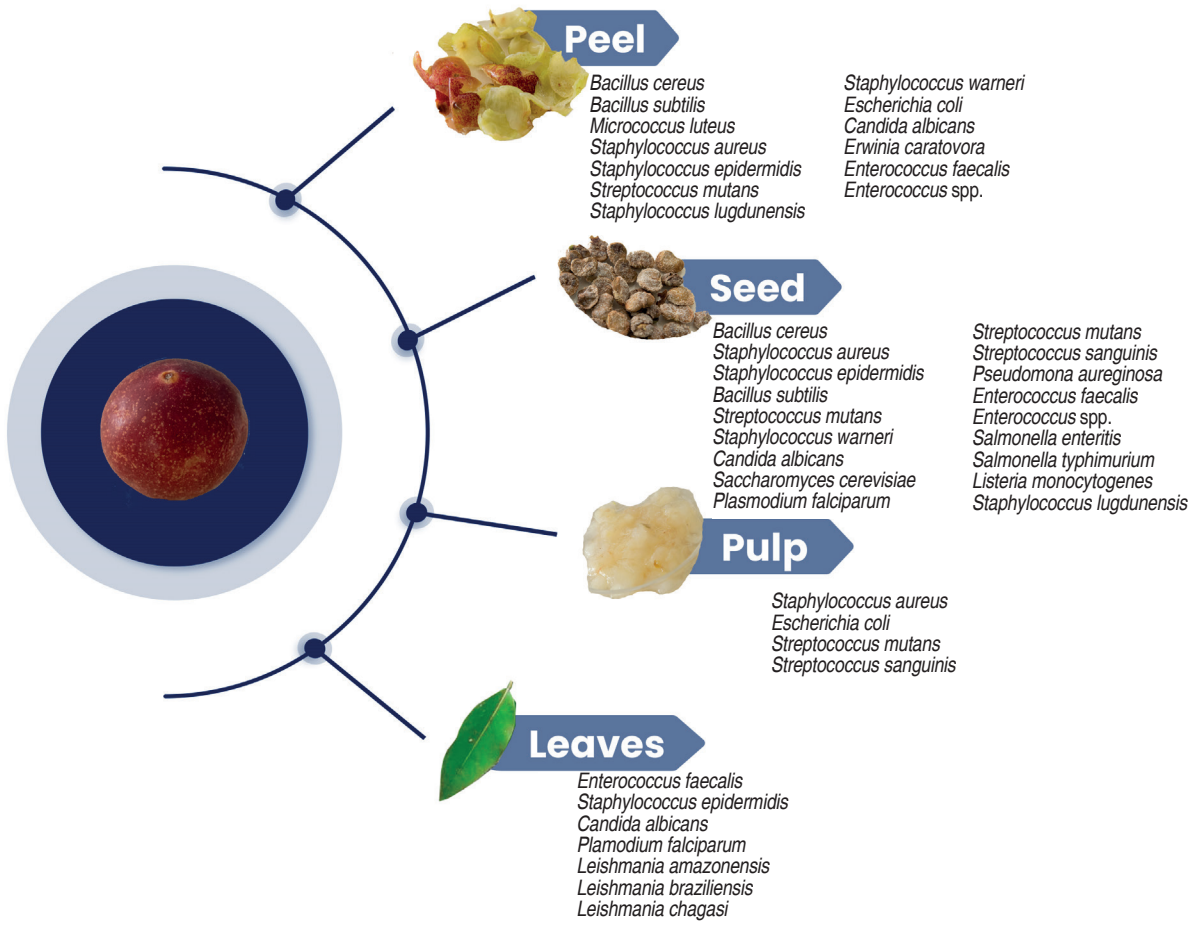

Figure 2. Microorganisms that can be inhibited by *M. dubia* (Kaneshima *et al*., 2017; Do Carmo *et al*., 2020; Fujita *et al.* 2015).

***M. dubia* against different microorganisms.**
Streptococcus mutans and *S. sanguinis* were inhibited using 100 µL of methanolic extracts from *M. dubia* pulp and seed. For both *S. mutans* and *S. sanguinis*, *M. dubia* seed extract had a major antibacterial (with inhibition zones of 21.36 and 19.21 mm, respectively) effect compared with the pulp extract. The MIC of methanolic seed extract against both strains could not be determined due to antibacterial activity even at very low concentrations of the extract. However, for the pulp extract, a MIC value of 62.5 µg mL^{-1} was observed for both strains (Camere-Colarossi *et al*., 2016). The use of

hydroethanolic extracts of *M. dubia* at concentrations of 25, 50, and 75 mg mL^{-1} on antibacterial activity *in vitro* for *S. mutans* ATCC 35668 was evaluated, evidencing an increase in antibacterial activity directly proportional to the concentration of the extract. The concentration of 75 mg mL^{-1} presented an average inhibition of 18.2±0.774 mm, followed by the concentration of 50 mg mL^{-1} with an average inhibition of 14.6±1.055 mm and the concentration of 25 mg mL^{-1} with an average inhibition of 10.1±0.833 mm. The zone of inhibition of the positive control was 16.5±0.516 mm, probably rhodomyrtone is responsible for the antibacterial activity since in addition to being present in the peel and seeds it is also found in the pulp (Ruiz-Barrueto *et al*., 2021).

Similarly, some authors demonstrated the inhibition of *Erwinia carotovora subsp. carotovora* by *M. dubia*, the following peel extracts revealed that 50% acetonic extract presented high inhibition, followed by ethanolic extract (50%), and chloroform extract (50%). For *Pseudomonas cichorium*, ethanolic extract (50%) presented greater inhibitory capacity followed by acetonic and chloroformic extracts (Flores and Naupari, 2017).

The methanolic extract of *M. dubia* seed showed activity against *P. aureginosa* ATCC25783 with MIC values of 1.2 mg mL^{-1} while *P. aureginosa* 8131 had no activity. On the other hand, the methanolic extracts of peel, seed and leave of *M. dubia* showed activity against *Enterococcus faecalis* T25-17 with MIC values of 0.3 mg mL^{-1} (peel and leave), 1.2 mg mL^{-1} (seed); in the same way, for *Enterococcus* spp. 8153 with MIC values of 0.3 mg mL^{-1} (peel and seed) (Roumy *et al*., 2020).

In another study, Nile tilapia (*Oreochromis niloticus*) tests were carried out on fish supplemented with 500 mg of *M. dubia* per kilogram of feed, it was observed a greater immune response of the fish against *Aeromonas hydrophila* in their swim bladder. The high ascorbic acid content of this plant increases the activity of leukocytes against pathogens and makes neutrophils in the blood increase and migrate to the site of infection to recognize and destroy pathogens, as well as the number of lymphocytes that generate antibodies. Furthermore, lysozyme serum exhibits the ability to hydrolyze peptidoglycans from the cell wall of pathogens (Yunis-Aguinaga *et al*., 2016).

Additionally, the B rings of the flavonoids interact with the hydrogens of the nucleic acids, inhibiting their synthesis; others can act at the cellular level of the bacteria, causing the release of components that can inactivate the bacteria (Cushnie and Lamb, 2005).

In another study, the optimized lyophilized *M. dubia* seed extract (1:20 g:mL of the mixture of 43.3% propanone, 40.7% water, and 16% ethyl alcohol) inhibited *P. aeruginosa* IAL1853 (8.72 mm), *S. enteritidis* S 2887 (6.82 mm), *S. typhimurium* IAL2431 (6.42 mm), *B. cereus* ATCC14579 (9.04 mm), *Listeria monocytogenes* ATCC7644 (8.58 mm) (Fidelis *et al*., 2020). However, Da Silva *et al*. (2021) studied the level at which *M. dubia* powder 0.0, 2.0, 3.5, or 5.0% (w/w), mixed with 200 g of ground meat and *Salmonella enterica* ser. *typhimurium* (5 log CFU g^{-1}). The concentration of CPP at 5% had an inhibition value of 5.089 log UFC g^{-1} *S. enterica* compared to control without CPP (5.121 log UFC g^{-1} *S. enterica*), indicating the rapid decrease in the concentration of *Salmonella* when increasing the concentration of CPP by interfering with the adaptability of the pathogens; however, it does not extend the shelf life of ground meat.

Furthermore, Willemann *et al*. (2020) showed that 2 mg of lyophilized aqueous extract of camu camu seed exocarp inhibited the growth of *L. monocytogenes* (11.9 mm), *P. aeruginosa* (8.9 mm), *S. typhimurium* (8.9 mm), *S. enteritidis* (10.5 mm) and *B. cereus* (8.8 mm).

CONCLUSIONS

Phenolic compounds of *M. dubia* (peel, pulp, seeds, and leaves) such as polyphenols, flavonoids, and anthocyanins have been studied and categorized as responsible for the inhibition of different Gram-positive bacteria (*L. monocytogenes, S. aureus*), Gram-negative bacteria (*E. coli*, *S. typhimurium, S. enteritidis, P. aeruginosa, S. tiphy*), yeasts (*S. cerevisiae, C. albicans*), protozoa (*P. falciparum, L. amazonensis, L. braziliensis, L. chagasi*) and other pathogenic microorganisms that could affect food, whose action could be due to functional interference of bacterial enzymes in their structures, bacteriostatic action on ribosomal function or protein synthesis and blocking of RNA or DNA synthesis by catalytic inhibition of DNA gyrase. The inhibition of protozoa is possibly due to the action of quercetin in causing mitochondrial dysfunction in these parasites.

The inhibitory capacity of *M. dubia* extracts might not affect beneficial probiotic bacteria and could be applied in foods after further studies on the subject.

Further fractionation and purification studies of compounds present in the different parts of *M. dubia* and evaluated against pathogenic and food spoilage microorganisms are required. It is also necessary to explain the mechanism of action of inhibition of the different compounds at the cellular level.

CONTRIBUTION OF THE REVIEW

Information regarding the antimicrobial capacity of *M. dubia*, an Amazonian fruit from countries such as Peru, Brazil, Colombia, and Venezuela, has been identified and organized, offering a possible alternative to be used as an antimicrobial additive in the food industry after further studies.

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Chemical and structural changes of ocote pine (*Pinus oocarpa*) wood caused by thermal modification

Cambios químicos y estructurales de la madera de pino ocote (*Pinus oocarpa*) causados por la modificación térmica

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ABSTRACT

Thermal modifications alter the physical properties and improve the natural durability of wood without using chemical impregnation processes being an environmentally friendly alternative; these improvements could be made due to changes in the internal wood structure. In this investigation, changes caused to the chemical composition and microstructure of wood *Pinus oocarpa* by thermal modification at 170 and 190 °C were evaluated. The thermal treatment was carried out in a prototype chamber operated during the modification steps under a steam atmosphere, with a continuous flow without pressure. The evaluation of chemical changes was performed following the TAPPI standards. Using Fourier transform infrared spectroscopy (FTIR) and optical microscopy, the wood microstructure was characterized. The results showed a decrease in holocellulose contents by 7% at 170 °C and by 9% at 190 °C; lignin contents increased at 170 °C (6%) and at 190 °C (8%) and extractives were increased by 8% (170 °C) and 13% (190 °C); the changes obtained by the spectra were related to the C-H deformation in cellulose and hemicelluloses and the complex bonds of lignin carbohydrates of the -CH_3 (lignin) and CH_2 (carbohydrates) groups and organic acids released during thermal modification. Thickness of tracheid walls decreased in earlywood 8% (170 °C) and 22% (190 °C) and in latewood 11% (170 °C) and 14% (190 °C); lumen diameters increased in earlywood to 170 °C (14%) and 190 °C (48%) and in latewood in 14% (170 °C) an in 20% (190 °C). At 190 °C, the cell alterations were higher. *Pinus oocarpa* was thermally modified at 170 °C showing better wood quality in its internal structure.

Palabras clave:
Madera anatómica
Composición química de la madera
Tratamiento térmico
Modificación de madera

RESUMEN

La modificación térmica aumenta las propiedades físicas y durabilidad natural de la madera sin adición de procesos químicos de impregnación, siendo amigable con el ambiente; dichas mejoras se deben a los cambios ocasionados en la estructura interna de la madera. Por ello, la presente investigación evaluó los cambios sobre la composición química y microestructura de la madera de *Pinus oocarpa* por la modificación térmica a 170 °C y 190 °C realizada en un cámara prototipo que funciona durante los pasos de modificación bajo una atmósfera de vapor de agua, con un flujo continuo de corriente sin presión. La evaluación de los cambios químicos se realizó bajo las normas TAPPI. Los cambios microestructurales fueron evaluados usando Fourier transform infrared spectroscopy (FTIR) y microscopia óptica. Los resultados mostraron disminución de los contenidos de holocelulosa en 7% (170 °C) y 9% (190 °C); los contenidos de lignina aumentaron para 170 °C (6%) y a 190 °C (8%) y los extractos aumentaron un 8% (170 °C) y 13% (190 °C). Los cambios presentados en los espectros se relacionaron con la degradación C-H en celulosa y hemicelulosas y los enlaces complejos de lignina y carbohidratos de los grupos -CH_3 (lignina), -CH_2 (carbohidratos) y los ácidos orgánicos liberados durante la modificación térmica. El espesor de las paredes de las traqueidas disminuyó en madera temprana 8% (170 °C) y 22% (190 °C) y en la madera tardía 11% (170 °C) y 14% (190 °C); los diámetros de los lúmenes aumentaron en la madera temprana a 170 °C (14%) y a 190 °C (48%) y en la madera tardía en 14% (170 °C) y en 20% (190 °C). A una temperatura de 190 °C fueron mayores las alteraciones celulares. El tratamiento a 170 °C mostró una madera de *Pinus oocarpa* de mejor calidad en su estructura interna.

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Timber from tropical forest species is depleting at a fast rate due to industrialization. Therefore, it is necessary to use wood from fast-growing forest plantation species to be used in different wood applications. However, the wood of such species is often characterized by different problems such as low dimensional stability, low mechanical properties, and low natural durability against bio-deteriorating agents, which makes them undesirable for many economic purposes (Shukla, 2019; Unsal *et al*., 2011); as is the case of the wood of the pine ocote *Pinus oocarpa* Shiede ex Schltdl. It presents a high dimensional instability, low mechanical resistance, and it is very susceptible to being attacked by biological agents, which could cause some limitations in structural purposes. The above limitations of wood can be overcome using thermal modification, which is achieved by subjecting the wood to temperatures between 150 and 260 °C with an inert atmosphere or with low oxygen content for times ranging from a few minutes to several hours, depending on the species and the desired degree of modification (Sandberg *et al*., 2013; Esteves *et al*., 2014). The thermal modification process is an environmentally friendly treatment without the addition of toxic chemicals showing an improvement in the behavior of the properties of wood, with increases in dimensional stability, density, resistance to biodegradable agents and color change, a very important characteristic for many uses considered as a relevant attribute for marketing, which provides value-added for low-quality woods to substitute certain high-value tropical species (Esteves *et al*., 2014; Hermoso *et al*., 2015; Herrera-Builes *et al*., 2021; and Kubovský *et al*., 2020).

In most studies on thermal treatments, independent of the species used, there are alterations in the mechanical properties; and the treatment temperature is determinant in the results obtained (Frühwald, 2007).

The new internal properties resulting from thermally modified wood are associated with the degradation of the structure of the cell wall polymers and from the formation of modified extractives, which occur due to changes in the composition of the wood through different chemical reactions (Esteves *et al*., 2014; Kubovský *et al*., 2020). In addition, changes in the anatomical structures of wood depend on the wood species and the processing method and conditions used (Boonstra *et al*., 2006).

In most studies about thermal modification, independent of the wood species considered, there are alterations in the chemical composition, and the heat treatment is determinant in the results. Thus, when applying heat treatments between 170 °C and 190 °C under steam pressure, there is a decrease in holocellulose content caused by its degradation; but, due to said such thermal degradation, lignin and extractives increase because of the formation of carbohydrate conversion products in the modified wood of specimens of *Pinus sylvestris*, *Pinus brutia* and *Pinus massoniana* (Boonstra MJ and Tjeerdsma, 2006; Durmaz *et al*., 2019; Ates *et al*., 2009; Wang *et al*., 2018). However, for *Pinus oocarpa* themally modified significant differences in chemical components were detected just from 200 ºC temperature (Carvalho *et al*., 2020).

The changes in anatomical structures of wood caused by thermal modification were evaluated by Jang and Kang (2019) using *Pinus densiflora* wood, which was subjected to heat treatment at temperatures of 190, 210, and 230 °C; after performing the analyzes, changes were found in the microstructure with cell walls thinned and with an increase in the lumen diameter of the treated wood. For *Pinus radiata* is suggested that these changes on the tracheid cell walls could be minimized, improving the time-temperature schedules during the process of wood thermal modification (Cabezas-Romero *et al*. 2021). Bernabei and Salvatici (2016) investigated the response to the anatomical structural changes in the wood *Picea abies* modified thermally, reporting changes in the tracheid wall thickness and lumen diameter above 200 °C. When treating *Thuja plicata* wood at 220 °C, increases in lumen diameter and destruction of its tracheid wall were obtained (Awoyemi and Jones, 2011). Effects of thermal modifications at 165 and 185 °C on woods of *Pseudotsuga menziesii, Pinus sylvestris, Picea abies* and *Pinus radiata* have been noticed, such as small cracks between tracheids with curved and severed deformations of the lumens (Boonstra *et al*., 2006).

For *Pinus oocarpa* wood, thermal modification was shown to improve some physical properties and static bending (Herrera-Builes *et al*., 2021). This study aimed to analyze the effect of thermal modification on the internal structure and chemical composition of *Pinus oocarpa* wood, in order to contribute to the knowledge and characterization of this kind of important forest plantation species.

MATERIALS AND METHODS

Materials

Sawn wood from *Pinus oocarpa* Schiede ex Schltdl var. Ochoterenai was used for the heat treatment experiments, provided by the Empresa Cipreses de Colombia S.A., from mature trees (25 years old) collected from plantations located in the municipality of Yolombó (Antioquia, Colombia) coordinates 6°39´25.4" N 75°04´13.9" W. A total of 30 trees were collected randomly, with a useful shaft with a diameter of 450 mm, from which the first two logs of 2500 mm long were taken, a central plank of 100 mm thick heartwood was cut from each log; then from each plank, slats of 60×60×2500 mm (width, thickness, and length, respectively) were cut, eliminating the pith, which was subjected to air-dry until reaching a moisture content of 12% and samples of 410 mm (Longitudinal) × 25 mm (Tangential) × 25 mm (Radial) were taken.

Thermal modification process

The thermal modifications of 170 °C and 190 °C to which the wood *Pinus oocarpa* was submitted were made in a prototype flexible oven chamber with a capacity of 3.5 m^3 designed for temperatures up to 250 °C (Neumann, Model Lab3.5e, Concepción, Chile). For each treatment, the chamber was loaded with 120 boards of 50 mm wood, which were stacked evenly and placed in layers of equal distance, with 20-mm-thick spacers to allow the steam to move through the pile and distribute the weight vertically from top to bottom; the *Pinus oocarpa* specimens were stacked inside the middle of the woodpile. They were continuous and without empty spaces to prevent oxygen currents. The prototype chamber operated during the modification steps was under an atmosphere of steam, with a continuous flow without pressure. The dry and wet bulb temperatures, as well as the wood temperature, were monitored according to the configuration and the computerized kiln program (Canelo, Control Total, v8.0, Concepción, Chile). Specifically, the modification process began with a temperature increase rate of 1 °C min^{-1} up to 100 °C. The temperature was then maintained for 15–22 h, allowing the wood to dry 4–6% of the moisture content. Subsequently, a temperature increase rate of 0.7 °C min^{-1} was applied up to 170 or 190 °C. In this step, a steam atmosphere was used to reduce air and prevent damages. The treatment temperature (170–190 °C dry bulb or 100 °C wet bulb) was maintained for approximately 2.5 h. The last stage was the cooling and stabilization of the samples for approximately 5–7 h. The temperature gradient between the surface and the center of the samples did not exceed 15–20 °C, in order to maintain the quality of the wood. The treatment time was about 30 h, and the final moisture content of the specimens was 9–11%. Finally, two thermally modified loads (two programmed temperatures) were obtained, and the different samples of *Pinus oocarpa* were analyzed to characterize the heat-treated material and compare it with the unmodified wood material.

Determination of chemical composition

Samples of unmodified and thermally modified wood were grounded to sawdust. The material used for chemical analysis was classified between 40 and 60 mesh. It was extracted in the Soxhlet equipment with a mixture of ethanol and benzene according to the TAPPI T 204 CM-97 (1997).

The holocellulose content was determined using sodium chlorite according to the procedure of Wise *et al*. (1946). In a beaker, 150 mL of 1.5% (w/w) sodium chlorite solution were poured into g of wood, and 10 drops of glacial acetic acid and the sample was placed in a water bath at 75 °C for 1 h. After 1 h, 10 drops of glacial acetic acid and 1.5 g of sodium chlorite were added, it was cyclically repeated every hour, for a total period of 4 h. After chlorination, the solution was filtered, and washed with 200 mL of cold water, followed by 10 mL of acetone; the residue was taken to a conventional oven at 105 °C until constant weight.

The lignin content was determined as described by TAPPI T 222 om-02 (2002). 15 mL of 72% sulfuric acid (w/w) were added to 1 g of wood sample, stirring it for 10 min and letting it rest for 2 h. Subsequently, 575 mL of distilled water were added, boiled for 4 h maintaining a constant volume, if necessary distilled water was occasionally added. Finally, it was filtered in Büchner funnels, and the samples were repeatedly washed until the acid residues were removed. Finally, they were brought to constant weight in an oven at 105 °C. All measurements were made in 12 replicates for each chemical composition determination. Data were presented as percentages on a dry basis.

FTIR spectroscopy

The thermally modified and the unmodified wood were studied using the spectroscopy of the Fourier Transform

Infrared (FTIR) technique. The spectra were obtained using a Shimadzu spectrophotometer (model IRTracer-100, Japan) with a range of 12500 and 240 cm^{-1} with a resolution of 4 cm^{-1}; applying the Attenuated Total Reflectance (ATR) method in wood samples of 10 mm (width) × 10 mm (thickness) × 20 mm (length).

Anatomical measurements

Pieces of samples of unmodified and thermally modified wood were cut into lengths of 20×20×20 mm and soaked in tap water for 36 h. A transverse slice of 20 µm was obtained using a Spencer Model 860 microtome (AO Instrument CO, Buffalo, N.Y, USA). These sections were stained with a 1% safranin solution and mounted on a glass slide and cover glass using Canada balsam and glycerin. Anatomical measurements were performed at the Forest Products Laboratory of the Universidad Nacional de Colombia, Medellín, Colombia. The changes in the cellular structure of wood were examined using a Primo Star light microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). All measurements were made in triplicate. The cell wall thickness and lumen diameter for early and latewood were measured in 12 measurements per sample from heat-treated and unmodified *Pinus oocarpa* wood (Figure 1).

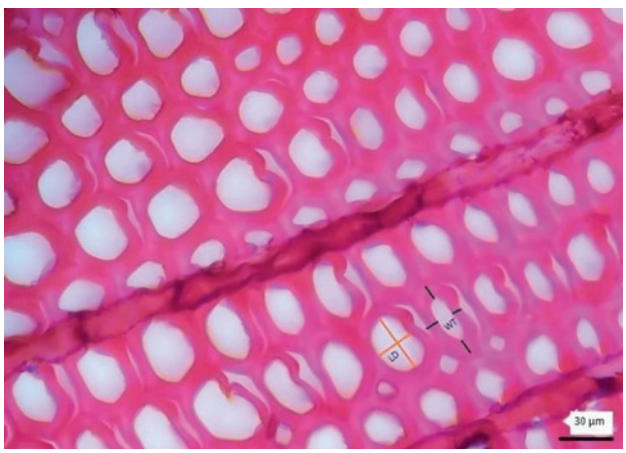

Figure 1. Unmodified *Pinus oocarpa* wood transverse surface of latewood 40x. measurements LD: lumen diameter; WT: wall thickness.

Data analysis

Shapiro-Wilk and Barlett's test were performed to verify the normality of data distribution and homogeneity of variance among treatments. Analysis of variance (ANOVA) was performed for each variable, and when the F test showed significant results, a Tukey multiple range test (P<0.05) was performed using the Statgraphics Centurion XVI statistical program. The information was processed based on the methodology described by Gómez (1989).

RESULTS AND DISCUSSION

Chemical composition

Thermally modified *Pinus oocarpa* wood presented a higher number of extractives and insoluble lignin than unmodified wood, in addition to presenting lower contents in the polysaccharides formed by holocellulose, also for modified wood (Table 1); the holocellulose content decreased by 7% in the wood treated at 170 °C, similar to those obtained by Durmaz *et al*. (2019) and Wang *et al*. (2018) in *Pinus sylvestris* and *Pinus massonica* woods; and for thermal modification, at 190 °C decreases of 9% were detected, equal to those achieved by other authors in heat-treated wood (Ding *et al*., 2011), no significant differences were found between the modified treatments; while, significant differences were found for the treated and untreated wood. These results disagree with those reported by Carvalho *et al*. (2020) in *Pinus oocarpa* found less content of holocellulose but heat-modified wood showed a significant decrease of holocellulose only at temperatures above 200 °C. These decreases were due to the deacetylation and formation of acetic acid, which acts as a catalyst for depolymerization reactions and accelerates the degradation of polysaccharides (González-Peña and Hale, 2009).

Table 1. Chemical composition contents in thermally modified and unmodified *Pinus oocarpa* wood.

Treatment	Lignin (%)	Holocellulose (%)	Extractives (%)
Unmodified	28.56 ±1.11 a	68.57±1.56 a	5.35±0.15 a
170°C 2.5h	30.39±1.18 b	64.06±1.44 b	5.75±0.16 b
190°C 2.5 h	30.81±1.20 b	62.41±1.38 b	6.06±0.17 c

The values are expressed as the mean±standard deviation. Means with the same letter among the column do not differ statistically (P<0.05).

Decreases in the holocellulose contents of the wood *Pinus oocarpa* could generate a small increase (apparently not real) in the lignin contents (Batista *et al.,* 2016) by 6% for heat treatment at 170 °C, which is consistent with the results found by Boonstra *et al.* (2006) and Wang *et al.* (2018) on thermally modified *Pinus sylvestris* and *Pinus massonica* woods, and the lignin content increased about 9% after thermal modification at 190 °C; presenting no statistically significant differences (P>0.5); these results are comparable to those obtained for *Pinus sylvestris* and *Pinus massonica* woods (Ding *et al.,* 2011; Wang *et al*., 2018). These results show significant differences between treated and untreated wood contrary to the results obtained by Carvalho *et al.* (2020) in *Pinus oocarpa*, who found higher lignin content but in heat-modified wood showed significant increment only at temperatures above 200 °C. The increase in lignin content during thermal modification is caused by the condensation of by-products that occurs by the degradation of polysaccharides, which become trapped forming a repolymerization of lignin (Tumen *et al.,* 2010).

Heat treated *Pinus oocarpa* wood had a different effect on the content of the modified wood extractives, which was higher as the temperature increased during the heating process, with statistically significant differences between treatments (P<0.05); thus, for the modification at 170 °C increases of 8% were obtained, equivalent to the results obtained by Boonstra *et al.* (2006) and Wang *et al.* (2018); nevertheless, higher increases of extractives were achieved for the modification to 190 °C, which increased by 13%, equal to those obtained in other treated woods (*Pinus sylvestris* and *Pinus bruttia)* (Ding *et al.,* 2011; Ates *et al*., 2009). The increases in the mass content of the extractives were caused by the products formed due to degradation, mainly of the hemicellulose of the wood cell wall (Batista *et al.,* 2016; Esteves *et al.,* 2008; Kučerová et al., 2016). However, Carvalho *et al.* (2020) in *Pinus oocarpa* found a low content of extractives and the heat-treated wood showed extractives significative different only at temperatures above 200 °C, which is contrary to the results in extractives with significant differences in thermal treatments with 170 °C and 190 °C.

FTIR spectroscopy

Changes in band intensities or ratios of the ATR-FTIR spectra are shown in Figure 2; comparing heat treatments with wood without modifying the chemical structure of *Pinus oocarpa* change significantly; the spectral changes that occurred after heat treatment were greater in the treatment at 170 °C; as observed at the peak of 3383 cm^{-1}, which is typical of the stretching vibrations of O-H as a result of the decrease of free hydroxyl groups due to degradation of hemicellulose, providing hydrophobicity to the treated wood (Gérardin *et al.,* 2007; Akgül *et al*., 2006; Salcedo *et al.,* 2016). The band at 2923 cm^{-1} corresponds to the stretching vibration C-H that may be related to the presence of methylene (CH_2) and methyl (CH_3) (Wu *et al.,* 2021). The peak at 1650 cm^{-1} is related to stretching vibration of C=O caused by the deacetylation and excision of hemicellulose, resulting in the release of acetic acid and aromatic structures (Gérardin *et al.,* 2007; Akgül *et al.,* 2006; Salcedo *et al.,* 2016; Popescu *et al*., 2013; Wu *et al.,* 2021). In relation to the band at 1368 cm^{-1}, it is related to the C-H deformation in cellulose and hemicelluloses and the complex bonds of lignin carbohydrates from the -CH_3 (lignin) and -CH_2 (carbohydrates) groups (Missio *et al.,* 2015; Wu *et al*., 2021; Gérardin *et al*., 2007). On the other hand, the band of 1051 cm^{-1} has its origin in the C-O deformation of the holocellulose and the C-O-C symmetric stretching of ether dialkyl and C-H deformation of aromatic lignin, it was more intense in the wood heat treated at 170 °C, it suggests the excision of β-O-4 and the excision of methoxylates from lignin (Wu *et al*., 2021; Popescu *et al*., 2013; Bhuiyan *et al*., 2000).

Finally, at the 807 cm^{-1} band is evidenced the bending vibration outside the CH plane in lignin and organic acids, released during treatment and catalyze the hydrolysis of hemicellulose and cellulose chains (Nuopponen *et al*., 2003; Gérardin *et al.,* 2007; Wu *et al*., 2021).

Anatomical measurements

Table 2 shows the anatomical measured values of cell wall thickness and the lumen diameter for the earlywood and latewood of thermally treated and unmodified *Pinus oocarpa* wood.

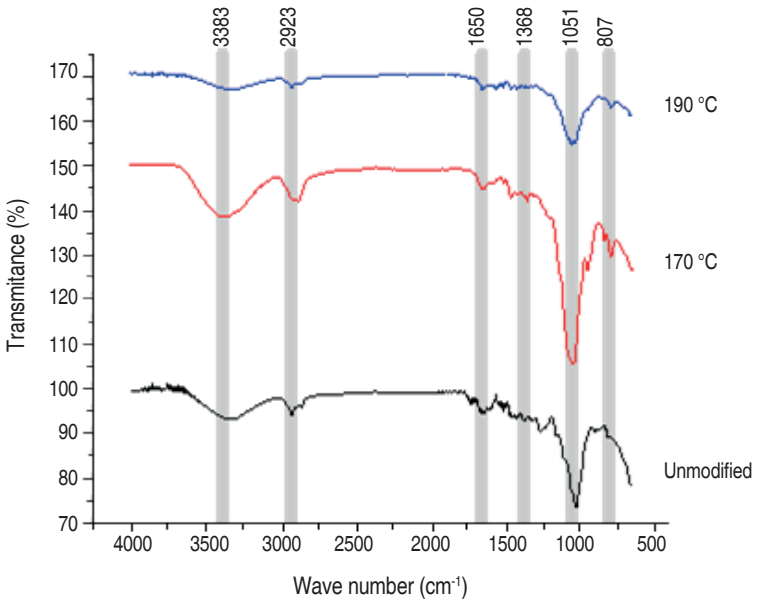

Figure 2. FTIR spectra of thermally modified *Pinus oocarpa* wood

According to the results, the cell wall thickness of the early wood decreased with the thermal modification of wood by 8% and 22% at 170 °C and 190 °C respectively, showing significant statistical differences between treatments in relation to cell wall thicknesses of latewood; also decreased with thermal modification by 11% for the thermal application of 170 °C and 14% for 190 °C, the difference between treatments was not statistically significant (P>0.05); the decreases obtained were higher than those reported in *Pinus radiata* and *Picea abies* woods (Cabezas-Romero *et al*., 2021; Bernabei and Salvatici, 2016).

The values of the cell lumen diameter resulting from the thermal modification in earlywood, increased by 14% after the application of the 170 °C treatment and 48% after treatment at 190 °C, clearly presenting statistically significant differences between treatments. On the other

Table 2. Anatomical measurements of cell wall thickness and the lumen diameter of the earlywood and latewood of heat-treated and untreated *Pinus oocarpa* wood.

Treatment	Cell wall thickness (µm)		Lumen diameter (µm)	
	Earlywood	Latewood	Earlywood	Latewood
Unmodified	3.51±0.14 a	4.50±0.16 a	12.31±0.88 a	8.59±0.72 a
170°C 2.5 h	3.24±0.15 b	4.00±0.14 b	14.04±0.59 b	9.81±0.66 b
190°C 2.5 h	2.74±0.16 c	3.85±0.11 b	18.31±0.89 c	10.33±0.71 b

The values are expressed as the mean±standard deviation. Means with the same letter among the columns do not differ statistically, Tukey's ($P \leq 0.05$).

hand, the modifications presented in the latewood were increased by 14% to 170 °C and 20% for 190 °C, without presenting significant statistical differences between the treatments.The above results were higher than those reported for *Pinus radiata, Pinus sylvestris* and *Picea abies* wood (Cabezas-Romero *et al*., 2021; Kekkonen *et al*., 2010; Bernabei and Salvatici, 2016).

Morphological changes in the anatomical structure of thermally treated *Pinus oocarpa* wood, compared to the unmodified condition are shown in Figure 3. Cracks and small voids between tracheids were noticed when applied the modification treatment at 170 °C (Figure 3A), in addition, crushing of the cell lumens and cell deformations were noted. Cabezas-Romero *et al*. (2021) reported for the modified *Pinus radiata* wood slight crushing of cellular lumens, small cracks and the presence of broken cells and also cell deformations. Through the thermal modification at 190 °C, an increase in the morphological changes of *Pinus oocarpa* wood was presented, which affects its use and potential assessment due to the presence of higher cell deformations, large cracks, as well as an increase of the breaking and destruction of the tracheid walls; a similar phenomenon was also reported by Awoyemi and Jones (2011) in *Thuja plicata* wood.

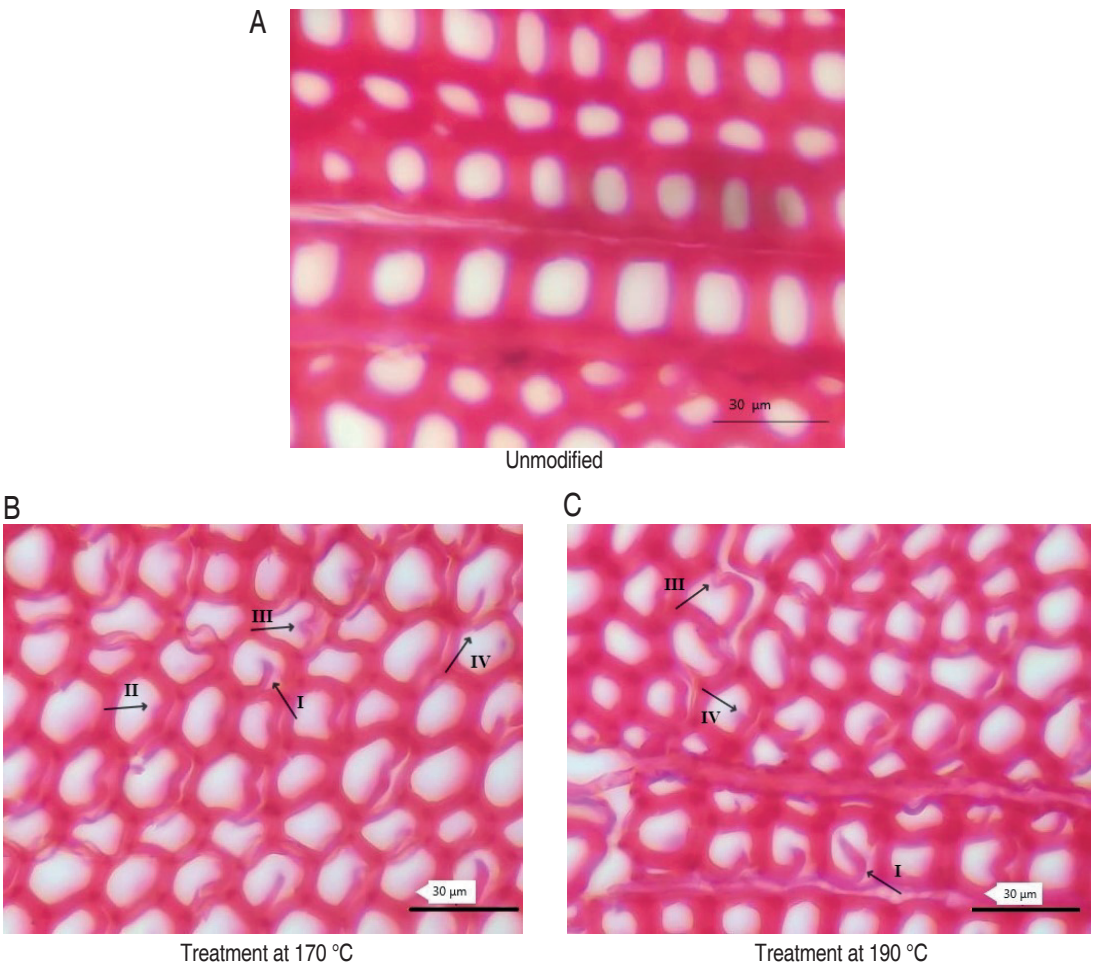

Figure 3. Morphological changes in the anatomical structure of the wood *Pinus oocarpa*. I: cracks; II: crushing; III: cell deformations and IV: voids.

CONCLUSIONS

The thermal modification caused changes in the chemical composition of the treated wood, with increases in lignin and extractives content, as well as a decrease in the holocellulose content (cellulose and hemicellulose). Thickness of tracheid walls decreased, and lumen diameter increased as the modification temperature increased. The treatment at 170 ° C produces better wood quality by obtaining lower degradation degrees and modification of the components of the cell wall. Future studies should focus on the resistance of treated wood to degradation, against the attack of fungi and xylophagous insects, because of chemical modifications found in the decrease of the carbohydrates.

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Los artículos deben ser enviados a través del Open Journal System en el Portal de Revistas de la Universidad Nacional de Colombia http://www revistas.unal.edu.co/. Sólo serán considerados artículos escritos en inglés. Adjunto se deben remitir los siguientes cuatro formatos: (1) Lista de verificación de criterios editoriales para la presentación de manuscritos; (2) Autorización de publicación de manuscritos en la Revista Facultad Nacional de Agronomía Medellín, en la cual se acepta la no postulación simultánea del artículo a otras revistas u órganos editoriales y se ceden los derechos a la Revista para su difusión, este debe ser firmado por todos los autores del manuscrito; (3) Datos personales de cada autor; (4) Sugerencia de posibles pares evaluadores. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión y artículos cortos. Los artículos pueden ser elaborados por profesores y/o investigadores de la Universidad Nacional de Colombia, o cualquier otra institución afín, nacional o internacional, en los temas Agropecuarios, Forestales y de Ingeniería Agrícola y de Alimentos. El manuscrito no debe exceder 5200 palabras para artículos de investigación y 6000 para artículos de revisión. Las hojas deben ser tamaño carta, escritas a interlineado doble, numeración de línea continua, letra o fuente Times New Roman o Verdana, tamaño 12 puntos, márgenes de 3 cm en la parte superior, 2 cm en la inferior y 2,5 cm en las márgenes laterales derecha e izquierda. Las tablas y figuras (es decir, los gráficos, dibujos, esquemas, diagramas de flujo, fotografías y mapas) se deben mostrar incorporadas en el texto y con numeración consecutiva (Tabla 1... Tabla n; Figura 1... Figura n, etc.). Los textos y tablas se deben presentar en el procesador de palabras MS-Word®;las tablas y los diagramas de frecuencia (barras y tortas) originales se deben suministrar en el archivo del documento y también en su original de MS-Excel®; otras figuras, como fotografías sobre papel y dibujos, se pueden enviar en original o escaneadas y ser remitidas en el formato digital de compresión JPG (o JPEG) preferiblemente con una resolución de 600 x 600 dpi (mínimo 300 dpi); es deseable que las fotografías originales sean enviadas como diapositivas. Como norma general, las tablas y figuras sólo se aceptan en blanco y negro; excepcionalmente se incluirán en color cuando sea estrictamente necesario y a juicio del Comité Editorial.

Unidades, abreviaturas y estilo

Se debe utilizar el Sistema Internacional de Unidades (SIU), y aquellas unidades específicas de mayor uso por parte de la comunidad científica. Las unidades combinadas deben usar la forma exponencial. Ejemplo: kg ha^{-1}. El significado de las abreviaturas debe citarse por extenso cuando se mencionan por primera vez en el manuscrito. El estilo de escritura debe ser absolutamente impersonal, en tiempo gramatical pasado para la introducción, los procedimientos y los resultados y presente para la discusión, evitando la conjugación de verbos en primera o tercera persona del singular o el plural.

Los números del uno al nueve se escriben en palabras, excepto cuando incluyen unidades de medida o se mencionan varios números. Ejemplo: “ocho tratamientos”, “3, 7 y 9 lecturas”, “15 kg”. Use cero antes del punto decimal. Para separar números en intervalos de uno o más años, use la letra “a”, y guión para temporadas de crecimiento. Ejemplo: Periodo 2002 a 2005; temporadas de crecimiento 1999-2000, 2000-2001.

Título y autores

El título del artículo no debe incluir abreviaturas y es obligatoria su respectiva traducción al idioma español. En lo posible, el título no debe exceder de 15 palabras y debe reflejar con precisión el contenido del documento. Cuando contenga nombres científicos de especies vegetales o animales, éstos se deben escribir con letra cursiva (itálica) en minúsculas, sólo con mayúsculas la primera letra del género y del clasificador. Debajo del título en inglés se escribe el nombre(s) y apellido(s) de los autores, sin sus respectivos títulos académicos, ni cargos laborales, en una línea horizontal y de acuerdo con su contribución en la investigación y/o preparación del artículo.

Como nota al pie de la primera página, se escribe el título de pregrado, el cargo laboral de los autores, el nombre y la ciudad de ubicación de la entidad a la cual prestan sus servicios o del patrocinador para la realización del trabajo y su respectiva dirección de correo electrónico, indicando el autor de correspondencia. Además, se debe adjuntar un resumen de la hoja de vida de los autores, donde se mencionen los artículos publicados en otras revistas.

Resumen, abstract y palabras claves

El resumen no debe exceder de 250 palabras escritas en un único párrafo. Se debe escribir en inglés y español. Debe contener en forma breve la justificación, los objetivos, los métodos utilizados, los resultados obtenidos más relevantes y las conclusiones. Es obligatorio acompañar el resumen con un máximo de seis palabras clave distintas a las utilizadas en el título. Se aceptan como palabras clave no sólo las palabras simples, sino también términos compuestos hasta de tres palabras. Deben ir escritas en minúsculas y separadas por comas.

Introducción

Puede tener o no título. Define el problema e informa sobre el estado del arte respecto al tema principal del artículo; además, señala las razones que justifican la investigación y plantea los objetivos de la misma. Es obligatorio acompañar los nombres vulgares con el nombre(s) científico(s) y la abreviatura(s) del clasificador en la primera mención dentro del texto. No se deben mencionar marcas de productos, sino su nombre genérico o químico

Materiales y métodos

En este apartado se deben describir en forma clara, concisa y secuencial, los materiales (vegetales, animales, implementos agrícolas o de laboratorio) utilizados en el desarrollo del trabajo; además, se mencionan los aspectos relacionados con la ubicación, preparación y ejecución de los experimentos. Se debe indicar el diseño seleccionado, las variables registradas, las transformaciones hechas a los datos, los modelos estadísticos usados y el nivel de significancia empleado. Evitar detallar procedimientos previamente publicados.

Resultados y discusión

Son la parte central del artículo, deben estar respaldados por métodos y análisis estadísticos apropiados. Se deben presentar de manera lógica, objetiva y secuencial mediante textos, tablas y figuras; estos dos últimos apoyos deben ser fáciles de leer, autoexplicativos y estar siempre citados en el texto. Las tablas se deben elaborar con pocas columnas y renglones. Se debe tener la precaución de incluir el nivel de significancia estadística representado por letras minúsculas del comienzo del alfabeto (a, b, c, d,...), un asterisco simple (*) para $P<0,05$, doble asterisco (**) para $P<0,01$ o triple asterisco (***) para $P<0,001$. Las investigaciones que no siguen un diseño estadístico, deben mostrar la información de manera descriptiva. Use subíndices para modificaciones, reserve superíndices para potencias o notas al pie en tablas y figuras.

La discusión: Se refiere al análisis e interpretación objetiva de los resultados, confrontándolos con los obtenidos en otras investigaciones, o con los hechos o teorías conocidos sobre el tema. Explica los resultados en particular cuando difieren de la hipótesis planteada. Destaca la aplicación práctica o teórica de los resultados obtenidos y

las limitaciones encontradas. Resalta la contribución que se hace a una determinada área del conocimiento y el aporte a la solución del problema que justifica la investigación. Finalmente, proporciona elementos que permitan proponer recomendaciones o lanzar nuevas hipótesis. No se deben hacer afirmaciones que van más allá de lo que los resultados pueden apoyar.

Conclusiones

Son las afirmaciones originadas a partir de los resultados obtenidos, deben ser coherentes con los objetivos planteados y la metodología empleada; además, expresar el aporte al conocimiento en el área temática estudiada y proponer directrices para nuevas investigaciones.

Agradecimientos

Si se considera necesario, se incluyen los agradecimientos o reconocimientos a personas, instituciones, fondos y becas de investigación, que hicieron contribuciones importantes en la concepción, financiación o realización de la investigación.

Formato de citación en el texto

- Se registra la fuente entre paréntesis, el cual debe incluir el apellido del autor y año, con coma entre autor y año. Ejemplo: (Pérez, 1995).

- Si hay más de una fecha se separarán con comas: Ejemplo: (Pérez, 1995, 1998, 2001)

- Si hay dos autores se citarán separados por la conjunción and. Ejemplo: (Gil and Ortega, 1993)

- Si hay varios trabajos de un autor publicados en un mismo año, se citarán con una letra en secuencia alfabética de los títulos, adosada al año. Ejemplo: (Gómez, 2000a, 2000b, 2000c)

- En el caso de citas con tres o más autores, es necesario mencionar en el texto el apellido del primero y reemplazar los demás por la expresión latina abreviada *et al.* (en cursiva) que significa y otros; en la referencia se deben poner los apellidos e iniciales de todos los autores. Ejemplo: (García *et al*., 2004).

- Cuando se hace referencia al autor dentro del texto, sólo se encierra el año entre paréntesis y se omite la coma que separa al autor del año. Ejemplo: (1) De acuerdo con Castañeda (2000), …; (2) Concorde con los resultados de Poveda *et al.* (2018) …

- Cuando es una cita de una cita se ponen la información de los autores citados y los autores citantes. Ejemplo: Magalhaes *et al.* (1979) expone que … (as cited in Gómez, 2004).

- Organizaciones se citan por sus siglas, en caso de no tener se cita con su nombre completo. Ejemplo: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

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Sólo se listan las referencias bibliográficas mencionadas en el texto. No se aceptan notas de clase o artículos en preparación, o cualquier otra publicación de circulación limitada.

Las referencias bibliográficas se deben ordenar alfabéticamente por el apellido del primer autor, sin numeración y sin sangría. Para citar varias publicaciones del mismo autor, se debe seguir el orden cronológico creciente; si son del mismo año, se debe seguir el orden alfabético de los títulos.

Las referencias deberán contener todos los datos que permitan su fácil localización. Las referencias se citan en el lenguaje de publicación.

En cada referencia para todos los autores cite primero el apellido, tener en cuenta que algunos autores hispanos citan sus dos apellidos, seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

Ejemplos:

Libros: Autor(es). Año. Título del libro. Edición. Casa editora, ciudad de su sede. Páginas consultadas (pp. #-#) o páginas totales (# p.). Ejemplo: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

Capítulos de libros: Autor(es). Año. Título del capítulo. Páginas consultadas (pp. # - #). En: Apellidos e iniciales de los compiladores o editores (eds.). Título del libro. Edición. Casa editora, ciudad de su sede. Páginas totales (# p.). Ejemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Artículos de revistas: Autor(es). Año. Título del artículo. Nombre completo de la revista volumen(número de fascículo): página inicial–página final. doi. Ejemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Ponencias en memorias de congresos, seminarios, simposios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Adam M. 1992. The Impact of the Common Agricultural Policy on Agriculture in Greece (Master's thesis). Cambridge University. Cambridge, United Kingdom. 80 p.

Cita de cita, sólo se referencia la fuente consultada. Ejemplo: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia.

Suplemento de revista: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Revista de Biología Tropical 52 Suppl. 2: 195-201.

Citas de internet: Autor(es). Año. Título del artículo. En: Nombre(s) de la publicación electrónica, de la página web, portal o página y su URL, páginas consultadas (pp. # - #) o páginas totales (# p.); fecha de consulta. Ejemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. In: Agricultura Tropical, http://agrotropical.edunet.es. 25 p. consulta: noviembre 2003.

Patentes: Autor(es). Año. Título. País de la patente y número. Fuente. Ejemplo: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

The Journal *Revista Facultad Nacional de Agronomía Medellín* (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia – Medellín. It is aimed at professors, researchers and students in agronomy, animal, and forestry sciences, food and agricultural engineering, agricultural advisers and at all those professionals who create knowledge and articulate science and technology to make the field more productive at business and rural economy levels.

The Journal receives and publishes, without any cost, research articles, reviews, revisions, letters to the editor and editorials written in the English language.

The Journal is a four-monthly publication at national and international level. Its aim is to publish original, unpublished, and peer-reviewed articles of a scientific nature which respond to specific questions and provide support and testing of a hypothesis, related to agronomy, animal husbandry, forestry engineering, food and agricultural engineering, and related areas that contribute to the solution of the agricultural constraints in the tropics.

Taking into account Colciencias (Administrative Department of Science, Technology and Innovation of Colombia) criteria, the journal welcomes papers of the following types:

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Review articles: Documents resulted from a completed research systematizing, analyzing, and integrating the published or unpublished research findings, on a field of science or technology, in order to report the progress and development trends. It is characterized by a careful review of the literature of at least 50 references. The maximum length must be 6000 words; excluding figures, tables, references. This type of articles is arbitrated and indexed.

Short articles: short paper presenting original preliminary or partial results of a scientific or technological research, which usually require a quick diffusion. In all cases 60% of references must come from articles published in the last ten years.

Articles must be submitted in accordance with the guidelines set forth in “Instructions to Authors”; those who violate the rules will not initiate the basic editorial process. Shall be filled the form “Authorization for Release of Works and Economic Rights Assignment”, which will be provided by the Journal. This document is explicit in mentioning that all authors are informed and agree with article submitted for consideration to the Journal, that there is no conflict of interest between them, and also state that the manuscript has not been and will not be submitted for publication to another Journal.

The Editorial Board, supported by a team of associate editors, will evaluate the scientific merit of the paper and will then submit it for evaluation under double-blind method- that is to say, strict anonymity in the review is kept- by two arbitrators specialized in the area, preferably one national and one international, who will give their report on the format provided by the Journal. The Editorial Board reserves the right to accept collaborations. The report, after the review process, can be: accepted for publication with no or few modifications; accepted for publication with major changes according to the comments of the evaluators; reconsidered for publication if it is substantially modified - in this case, it will be deemed as new material; rejected for publication. If articles are accepted, they will be returned to authors for correction and sent again to the Director of the Journal within 30 calendar days.

Printing of graphs, figures or photographs in color is optional and have an additional cost per page needed of hundred thousand Colombian pesos ($ 100,000). The editorial staff of the Journal reserves the right to make editorial changes in the text of the article (titles, abstracts, tables and figures). Authors will be consulted on changes whenever it is possible.

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INSTRUCTIONS TO AUTHORS

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Papers must be sent b through the Open Journal System in the Universidad Nacional de Colombia journals web side http://www.revistas.unal.edu.co/, Will be considered only papers written in English. The four following formats must be submitted with the manuscript: (1) Editorial Criteria Checklist for Paper Submission; (2) Paper Publishing Authorization for the Revista Facultad Nacional de Agronomía Medellín, which accepts no simultaneous nomination of the article to other journals or editorial bodies, and the rights are given to the Journal for its release by the signature of all the manuscript's authors; (3) Personal information of each author; (4) Suggestion of possible peer reviewers. Publishing forms are: scientific and technological research articles, review articles, reflection articles, and short articles. Articles can be developed by professors and/or researchers at the Universidad Nacional de Colombia, or other related national or international institution, on Agricultural, Forestry, Food and Agricultural Engineering matters. Article extension must not exceed 5,200 words for research articles and 6,000 words for reviews. The manuscript must be letter-size sheets, line spacing double, continuous line number 12 point Times New Roman or Verdana font, 3 cm margin at the upper, 2 cm in the lower, 2.5 cm on the left and right side margins. Tables and figures (i.e, graphics, drawings, diagrams, flowcharts, photographs and maps) should be shown on separate sheets and numbered consecutively (Table 1 ... Table n, Figure 1…Figure n, etc.). Texts and tables should be submitted in MS-Word® word processor, original tables and diagrams of frequency (bar charts and pie charts) must be supplied in manuscript file and in its original MS-Excel®; other figures, such as photographs on paper and drawings, can be sent in original or scanned and sent in digital format compression JPG (or JPEG), preferably with a resolution of 600 x 600 dpi (300 dpi at least); original photographs are suggested to be sent as slides. As a general rule, tables and figures are only accepted in black and white. Color figures will be exceptionally accepted when strictly necessary and under discretion of the Editorial Board.

Units, abbreviations and style

International System of Units (SI), and those specific units of greater use by the scientific community must be used. When required must be used the exponential form. Example: kg ha^{-1}. The meaning of abbreviations should be cited in full when first mentioned in the manuscript. The writing style should be totally impersonal. Introduction, procedures and results should be written in grammatical past tense. Discussion should be written in grammatical present tense, avoiding the conjugation of verbs in first or third person singular or plural.

The numbers from 1 to 9 are written in words, except when they include units of measure or several numbers are listed. Example: “eight treatments”, “3,7 and 9 readings”, “15 kg”. Use zero before the decimal point. To separate numbers in intervals of one to two years, use the letter “a” and hyphen for growing seasons. Example period 2002 to 2005, growing seasons 1999-2000, 2000-2001.

Title and authors

The article should not include abbreviations and its translation into English is required. As far as possible, the title should not exceed 15 words and must accurately reflect the paper content. When the article contains scientific names of plants or animals, they should be written in italics in lower case, only the first letter of gender and classifier should be capital. Under the title in English the author or authors' name (s) and surname (s) is /are written, without academic degrees or job positions, in a horizontal line according to the contribution to research and / or preparation of the article.

As a footnote on the first page, write the title of undergraduate, authors' job positions, the name and city location of the entity to which they serve, or the sponsors for the research work and their respective email address. In addition, a summarized authors' résumé including reference to the articles published in other magazines should be attached.

Abstract and key words

The abstract should not exceed 250 words written in a single paragraph. It must be written in English and Spanish. It should contain in brief the justification, aims, methods used, the most relevant results, and conclusions. It is required to accompany the abstract with a maximum of six key words, translated into English, different from those used in the title. Single words as well as compound terms of up to three words are accepted as key words. They must be written in lowercase, separated by commas.

Introduction

It may or not have a title. It defines the problem and reports on the state of the art on the main subject of the article, it also points out the reasons for the research and sets out its aims. It is required to accompany common names with the corresponding scientific name (s) name and abbreviation (s) of the classifier at the first mention in the text. Brands must not be mentioned but the generic or chemical name.

Materials and methods

In this section, materials (crops, livestock, agricultural or laboratory implements) used in the development of work should be clearly, concisely and sequentially described. Aspects related to the location, preparation and execution of experiments should also be mentioned. The selected design, the recorded variables, the changes made to data, the statistical models used and the significance level used should be indicated. Authors must avoid detailing procedures previously published.

Results

They are the central part of the article and must be supported by appropriate statistical methods and analysis. They should be presented in a logical, objective and sequential way through texts, tables and figures; the latter two supports should be easy to read, self- explanatory and always quoted in the text. The tables should be composed by few columns and rows. Care should be taken to include the statistical significance level represented by lowercase letters of the beginning of the alphabet (a, b, c, d,…), a single asterisk (*) for $P<0.05$, double asterisk (**) for $P<0.01$ or triple asterisk (***) for $P<0.001$. Researches that do not follow a statistical design should display the information in a descriptive way. Use subscripts to modifications, reserve superscripts for potencials or footnotes in tables and figures.

Discussion

It refers to the analysis and objective interpretation of results, comparing them with those obtained in other research, or with known facts or theories on the subject. It explains the results, especially when they differ from the stated hypothesis. It emphasizes the practical or theoretical application of the obtained results and constraints encountered. Discussion also highlights the contribution that is made to a particular area of knowledge and to the solution of the problem that justifies the research. Finally, it provides elements that allow making recommendations or launching new hypotheses. Statements that go beyond what the results may support should be avoided.

Conclusions

Conclusions are assertions arising from the obtained results. They should be consistent with the objectives stated and the methodology used. They should also express the contribution to knowledge in the studied subject area and propose guidelines for further researches.

Acknowledgements

If necessary, acknowledgements or recognitions to individuals, institutions, funds and research grants that made important contributions in the design, financing or carrying out of the research are included.

Citing in-text format

- Citations in the text should be in parenthesis and include author's surname and year, with comma in-between. Example: (Pérez, 1995).

- If more than one date, they are separated by commas: Example: (Pérez, 1995, 1998, 2001).

- If there are two authors, they will be separated by the conjunction and. Example: (Gil and Ortega, 1993)

- If there are several works of an author published in the same year, they will be cited with a letter in alphabetical sequence of titles, adjacent to year. Example: (Gómez, 2000a, 2000b, 2000c)

- For citations with three or more authors, it is necessary to mention in the text the surname of the first author and replace the others by the Latin expression *et al.* (in italics), which means and others. All authors should be mentioned in the reference. Example: (García *et al.*, 2004)

- When the author is referenced within the text, only the year is enclosed in parentheses, and the comma that separates the author from the year is omitted. Example: (1) According to Castañeda (2000), ...; (2) In accordance with the results of Poveda *et al.* (2018), ...

- When an indirect source is cited, the information of the cited authors and the citing authors are placed. Example: (Magalhaes *et al.* (1979) state that ... (as cited in Gómez, 2004).

- Organizations are cited by their initials; in case they do not have their full name is used. Example: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

References

Only bibliographical references cited in-text are listed in the references section. Lecture notes, articles in preparation, or any other publication with limited circulation are not accepted. Excessive self-citation should be avoided.

Bibliographic references are ordered alphabetically by first author's surname, without numbering and without indentation. To cite several publications of the same author, chronological increasing order must be followed. Alphabetical order of titles must be followed in case they are from the same year.

References should contain all the data allowing to its easy location. The titles of the papers, the surnames of the authors and the names of journals must be referenced and cited in their original language.

Examples:

For books: Author(s), Year. Book title, Edition, Publisher, Place of publication. Pages consulted (pp. #-#) or total pages.Example: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

For book chapters: Author(s). year. Chapter title. pages consulted (pp. # - #). In: Surnames and names of the editors or publishers (eds.). book title. Edition. Publisher, place of publication. total pages (# p.). Example: Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Beral H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

For journals: Author(s). year. Article title. journal full name volume(number): initial page-final page. Example: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Presentations in Memoirs of Congresses, seminars and symposia: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

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Theses and dissertations: Adam M. 1992. The impact of the common agricultural policy on agriculture in Greece (Doctoral dissertation). Cambridge University. Cambridge, United Kingdom. 80 p.

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Citation of a citation, list the secondary source in your reference list: Example: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Journal Supplement: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Journal of Tropical Biology 52 Suppl. 2: 195-201.

For internet citations: Author (s), year. Article. In: electronic publishing Name (s), the web page, portal or page name and its URL, pages consulted (pp. # - #) or total pages (# p.), date of consultation. Example: Arafat Y. 1996. Siembra de olivos en el desierto palestino. En: Tropical Agriculture, http://agrotropical.edunet.es. 25 p.; accessed: November 2003.

Patents: Author(s). Year. Title. Patent country and number. Retrieved from. Example: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

ÉTICA EN LA PUBLICACIÓN CIENTÍFICA Y ACUERDO SOBRE POSIBLES MALAS PRÁCTICAS

La revista Facultad Nacional de Agronomía espera y verificará que los autores, revisores, editores y en general la comunidad académica y científica involucrada en nuestro proceso editorial, sigan estrictamente las normas éticas internacionales requeridas en el proceso de edición.

La revista Facultad Nacional de Agronomía sigue las normas éticas presentes en el COPE Best Practice Guidelines for Journal Editors y por el International Standars for Editors and Authors publicado por Committee on Publication Ethics.

Los autores deben evitar incurrir al plagio de la información. La revista define los siguientes lineamientos, criterios y recomendaciones sobre la ética en la publicación científica:

1. Criterios generales[1]

1.1. Los artículos deben contener suficiente detalle y referencias que permitan replicar o rebatir el estudio.

1.2.Declaraciones fraudulentas o deliberadamente inexactas constituyen un comportamiento poco ético.

1.3. Si el estudio incluye productos químicos, procedimientos o equipos que tienen cualquier riesgo inusual inherente a su uso, el autor debe identificar claramente estos en el artículo.

1.4. Si el estudio implica el uso de animales o de seres humanos, el autor debe asegurarse que el artículo contenga una declaración que haga explícito que se realizaron todos los procedimientos de conformidad con las leyes y directrices institucionales.

1.5. Se deben respetar los derechos de privacidad de los seres humanos.

2. Autoría[2]

Criterios:

2.1. Un "autor" es la persona que ha hecho una contribución intelectual significativa al artículo, por lo tanto, todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que los reúnan deben ser mencionados de forma explícita.

2.2. Se deben cumplir colectivamente tres criterios básicos para ser reconocido como autor:

a) Contribución sustancial a la concepción y diseño, adquisición de datos, análisis e interpretación del estudio.

b) Redacción o revisión del contenido intelectual.

c) Aprobación de la versión final.

2.3. El orden de la autoría debe ser una decisión conjunta de los coautores.

2.4. Las personas que participen en un estudio pero que no se ajusten a los criterios de autoría deben aparecer como "Colaboradores" o "Personas reconocidas".

2.5. Hay tres tipos de autorías que se consideran inaceptables: autores "fantasma", que contribuyen sustancialmente pero no son reconocidos (a menudo pagados por promotores comerciales); autores "invitados", que no hacen ninguna contribución discernible pero se nombran para aumentar las posibilidades de publicación; y autorías "honorarias", que se basan únicamente en una afiliación tenue con un estudio.

Recomendaciones:

2.6. Antes de iniciar la investigación se recomienda documentar la función y la forma como se reconocerá la autoría de cada investigador.

2.7. No se debe mentir sobre la participación de una persona en la investigación o publicación, si su contribución se considerada "sustancial" se justifica la autoría, bien sea como coautor o colaborador.

2.8. No se debe asignar una autoría sin contar con el consentimiento de la persona.

2.9. Todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que reúnan los requisitos deben aparecer como autores o contribuidores.

2.10. Algunos grupos colocan los autores por orden alfabético, a veces con una nota para explicar que todos los autores hicieron contribuciones iguales al estudio y la publicación.

3. Cambios en la autoría[3]

Criterios:

3.1. Hace referencia a la adición, supresión o reorganización de los nombres de autor en la autoría de un artículo aceptado.

3.2. Las peticiones de añadir o eliminar un autor, o para reorganizar los nombres de los autores, deben ser enviados por el autor correspondiente del artículo aceptado, y deben incluir:

a) La razón por la cual debe ser añadido o eliminado, o los nombres de los autores reorganizado.

b) La confirmación por escrito (e-mail) de todos los autores que están de acuerdo con la adición, supresión o reorganización. En el caso de adición o eliminación de los autores,esto incluye la confirmación de que el autor sea añadido o eliminado.

4. Conflicto de intereses[4]

Criterios:

4.1. Cuando un investigador o autor, editor tenga alguna opinión o interés financiero/personal que pueda afectar su objetividad o influir de manera inapropiada en sus actos, existe un posible conflicto de intereses. Este tipo de conflictos pueden ser reales o potenciales.

4.2. Los conflictos de intereses más evidentes son las relaciones financieras, como:

a) Directas: empleo, propiedad de acciones, becas, patentes.

b) Indirectas: honorarios, asesorías a organizaciones promotoras, la propiedad de fondos de inversión, testimonio experto pagado.

4.3. Los conflictos también pueden existir como resultado de relaciones personales, la competencia académica y la pasión intelectual. Por ejemplo, un investigador que tenga:

a) Algún tipo de interés personal en los resultados de la investigación.

b) Opiniones personales que están en conflicto directo con el tema que esté investigando.

Recomendaciones:

4.4. Revelar si se está en algún conflicto real o potencial de intereses que influya de forma inapropiada en los hallazgoso resultados del trabajo presentado, dentro de los tres (3) años de haber empezado el trabajo presentado que podría influir indebidamente (sesgo) el trabajo.

4.5. Revelar el papel de un promotor (o promotores) del estudio, si los hubiere, en el diseño del estudio, en la recopilación, análisis e interpretación de los datos, en la redacción del informe y en la decisión de presentar el documento para su publicación.

4.6. Los investigadores no deben entrar en acuerdos que interfieran con su acceso a todos los datos y su capacidad de analizarlos de forma independiente, y de preparar y publicar los manuscritos.

4.7. Al presentar un documento, se debe hacer una declaración (con el encabezamiento "Papel que ha tenido la fuente de financiación") en una sección separada del texto y colocarse antes de la sección "Referencias".

4.8. Algunos ejemplos de posibles conflictos de intereses que deben ser revelados, incluyen: empleo, consultoría, propiedad de acciones, honorarios, testimonio experto remunerado, las solicitudes de patentes / registros y subvenciones u otras financiaciones.

4.9. Todas las fuentes de apoyo financiero para el proyecto deben ser revelados.

4.10. Se debe describir el papel del patrocinador del estudio.

5. Publicación duplicada[5]

Criterios:

5.1. Los autores tienen la obligación de comprobar que su artículo sea basado en una investigación original (nunca publicada anteriormente). El envío o reenvío intencional de su trabajo para una publicación duplicada se considera un incumplimiento de la ética editorial.

5.2. Se produce una publicación duplicada o múltiple cuando dos o más artículos, sin hacerse referencias entre sí, comparten esencialmente las

mismas hipótesis, datos, puntos de discusión y/o conclusiones. Esto puede ocurrir en diferentes grados: Duplicación literal, duplicación parcial pero sustancial o incluso duplicación mediante parafraseo.
5.3. Uno de los principales motivos por los que la publicación duplicada de investigaciones originales se considera no ético es porque puede dar lugar a una "ponderación inadecuada o a un doble recuento involuntario" de los resultados de un estudio único, lo que distorsiona las pruebas disponibles.

Recomendaciones:

5.4. Los artículos enviados para su publicación deberán ser originales y no deberán haberse enviado a otra editorial. En el momento del envío, los autores deberán revelar los detalles de los artículos relacionados (también cuando estén en otro idioma), artículos similares en prensa y traducciones.
5.5. Aunque un artículo enviado esté siendo revisado y no conozca el estado, espere a que la editorial le diga algo antes de ponerse en contacto con otra revista, y sólo si la otra editorial no publicará el artículo.
5.6. Evite enviar un artículo previamente publicado a otra revista.
5.7. Evite enviar artículos que describan esencialmente la misma investigación a más de una revista.
5.8. Indique siempre los envíos anteriores (incluidas las presentaciones de reuniones y la inclusión de resultados en registros) que pudieran considerarse una publicación duplicada.
5.9. Evite escribir sobre su propia investigación en dos o más artículos desde diferentes ángulos o sobre diferentes aspectos de la investigación sin mencionar el artículo original.
5.10. Se considera manipulador crear varias publicaciones a raíz de la misma investigación.
5.11. Si desea enviar su artículo a una revista que se publica en un país diferente o en un idioma diferente, pregúntaselo a la editorial si se puede hacer esto.
5.12. En el momento del envío, indique todos los detalles de artículos relacionados en un idioma diferente y las traducciones existentes.

6. Reconocimiento de las fuentes

Criterios:

6.1. Los autores deben citar las publicaciones que han sido influyentes en la determinación de la naturaleza del trabajo presentado.
6.2. Información obtenida de forma privada, no debe ser usada sin explícito permiso escrito de la fuente.
6.3. La reutilización de las tablas y / o figuras requiere del permiso del autor y editor, y debe mencionarse de manera adecuada en la leyenda de la tabla o figura.
6.4. La información obtenida en el transcurso de servicios confidenciales, tales como manuscritos arbitrales o las solicitudes de subvención, no debe ser utilizada sin el permiso explícito y por escrito del autor de la obra involucrada en dichos servicios.

7. Fraude científico[6]

Criterios:

7.1. El fraude en la publicación científica hace referencia a la presentación de datos o conclusiones falsas que no fueron generados a través de un proceso riguroso de investigación.
7.2. Existen los siguientes tipos de fraude en la publicación de resultados de investigación:
a) Fabricación de datos. Inventar datos y resultados de investigación para después comunicarlos.
b) Falsificación de datos. La manipulación de materiales de investigación, imágenes, datos, equipo o procesos.
La falsificación incluye la modificación u omisión de datos o resultados de tal forma que la investigación no se representa de manera precisa. Una persona podría falsificar datos para adecuarla al resultado final deseado de un estudio.

Recomendaciones:

7.3. Antes de enviar un artículo, lea cuidadosamente las políticas editoriales y de datos de la revista.
7.4. Nunca modifique, cambie u omita datos de forma intencional. Esto incluye materiales de investigación, procesos, equipos, tablas, citas y referencias bibliográficas.
7.5. Tanto la fabricación como la falsificación de datos son formas de conducta incorrecta graves porque ambas resultan en publicaciones científicas que no reflejan con precisión la verdad observada.
7.6. El autor debe hacer una gestión adecuada de los datos que soportan la investigación, teniendo especial cuidado en la recopilación, producción, conservación, análisis y comunicación de los datos.
7.7. Mantenga registros minuciosos de los datos en bruto, los cuales deberán ser accesibles en caso de que un editor los solicite incluso después de publicado el artículo.

8. Plagio[7]

Criterios:

8.1. El plagio es una de las formas más comunes de conducta incorrecta en las publicaciones, sucede cuando uno de los autores hace pasar como propio el trabajo de otros sin permiso, mención o reconocimiento. El plagio se presenta bajo formas diferentes, desde la copia literal hasta el parafraseado del trabajo de otra persona, incluyendo: datos, ideas, conceptos, palabras y frases.
8.2. El plagio tiene diferentes niveles de gravedad, como por ejemplo:
a) Qué cantidad del trabajo de otra persona se tomó (varias líneas, párrafos, páginas, todo el artículo)
b) Qué es lo que se copió (resultados, métodos o sección de introducción).
8.3. El plagio en todas sus formas constituye una conducta no ética editorial y es inaceptable.
8.4. La copia literal solo es aceptable si indica la fuente e incluye el texto copiado entre comillas.

Recomendaciones:

8.5. Recuerde siempre que es esencial reconocer el trabajo de otros (incluidos el trabajo de su asesor o su propio trabajo previo) como parte del proceso.
8.6. No reproduzca un trabajo palabra por palabra, en su totalidad o en parte, sin permiso y mención de la fuente original.
8.7. Mantenga un registro de las fuentes que utiliza al investigar y dónde las utilizó en su artículo.
8.8. Asegúrese de reconocer completamente y citar de forma adecuada la fuente original en su artículo.
8.9. Incluso cuando haga referencia a la fuente, evite utilizar el trabajo de otras personas palabra por palabra salvo que lo haga entre comillas.
8.10. El parafraseado solo es aceptable si indica correctamente la fuente y se asegura de no cambiar el significado de la intención de la fuente.
8.11. Incluya entre comillas y cite todo el contenido que haya tomado de una fuente publicada anteriormente, incluso si lo está diciendo con sus propias palabras.

9. Fragmentación[8]

Criterios:

9.1. La fragmentación consiste en dividir o segmentar un estudio grande en dos o más publicaciones.
9.2. Como norma general, con tal de que los "fragmentos" de un estudio dividido compartan las mismas hipótesis, población y métodos, no se considera una práctica aceptable.
9.3. El mismo "fragmento" no se debe publicar nunca másde una vez. El motivo es que la fragmentación puede dar lugar a una distorsión de la literatura haciendo creer equivocadamente a los lectores que los datos presentados en cada fragmento (es decir, artículo de revista) se derivan de una muestra de sujetos diferente. Esto no solamente sesga la "base de datos científica", sino que crea repetición que hace perder el tiempo de los editores y revisores, que deben ocuparse de cada trabajo por separado. Además, se infla injustamente el número de referencias donde aparece citado el autor.

Recomendaciones:

9.4. Evite dividir inapropiadamente los datos de un solo estudio en dos o más trabajos.
9.5. Cuando presente un trabajo, sea transparente. Envíe copias de los manuscritos estrechamente relacionados al manuscrito en

cuestión. Esto incluye manuscritos publicados, enviados recientemente o ya aceptados.

10. Consentimiento informado

Criterios:

10.1. Los estudios sobre pacientes o voluntarios requieren la aprobación de un comité de ética.

10.2. El consentimiento informado debe estar debidamente documentado.

10.3. Los permisos y las liberaciones deben ser obtenidos, cuando un autor desea incluir detalles de caso u otra información personal o imágenes de los pacientes y cualquier otra persona.

10.4. Especial cuidado debe tenerse con la obtención del consentimiento respecto a los niños (en particular cuando un niño tiene necesidades especiales o problemas de aprendizaje), donde aparece la cabeza o la cara de una persona, o cuando se hace referencia al nombre de un individuo u otros datos personales.

11. Corrección de artículos publicados[9]

Criterio:

Cuando un autor descubre un error o inexactitud significativa en el trabajo publicado, es obligación del autor notificar de inmediato a la revista y cooperar en el proceso de corrección.

Referencias

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

Elsevier. «Autoría. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

———. «Conflicto de intereses. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

———. «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

———. «Ethics. Conducting research». Accedido 8 de agosto de 2014. http://www.elsevier.com/journal-authors/ethics#conducting-research.

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———. «Fraude en investigación. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

———. «Plagio. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

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[1] Elsevier, «Ethics. Conducting research», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#conducting-research.

[2] Elsevier, «Autoría. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

[3] William Black, Rodolfo Russo, y David Turton, «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes», Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

[4] Elsevier, «Conflicto de intereses. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

[5] Elsevier, «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

[6] Elsevier, «Fraude en investigación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

[7] Elsevier, «Plagio. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

[8] Elsevier, «Fragmentación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updated updatedURL.pdf.

[9] Elsevier, «Ethics. Writing an article», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#writing-an-article.

PUBLICATION ETHICS AND PUBLICATION MALPRACTICE STATEMENT

The journal Revista Facultad Nacional de Agronomia follows the COPE Code of Conduct and Best Practice Guidelines for Journal Editors and the International Standards For Editors and Authors, published by Committe on Publication Ethics.
The journal puts forth the following criteria and recommendations for ethical scientific publications:

1. General criteria[1]

1.1. Articles must contain sufficient details and references that allow the study to be replicable or refutable.
1.2. Fraudulent or deliberately inexact statements constitute unethical behavior.
1.3. If a study includes the use of chemical products, procedures, or equipment that presents an inherent risk, the author must state so in the article.
1.4. If the study involves the use of animals or human beings, the article must contain a clear statement that all of the procedures were carried out in strict compliance with laws and institutional directives.
1.5. The privacy of the human beings must be respected.

2. Authorship[2]

Criteria:
2.1. An "author" is a person that has made a significant intellectual contribution to an article; all of the individuals that are named as authors must fulfill the requirements for authorship and all of those individuals that do so must be explicitly named.
2.2. Three basic criteria must be met in order to be considered an author:
a) Substantial contribution to the study concept, design, and data collection, analysis and interpretation.
b) Revision of the intellectual content.
c) Approval of the final version.
2.3. The order of the author list must be a joint decision of the coauthors.
2.4. The individuals that participate in a study but that do not meet the criteria for authorship must be listed as an "Assistant" or "recognized person."
2.5. There are three types of unacceptable authorship: "ghost" authors, who make a substantial contribution but are not recognized (often paid by commercial promoters); "guest" authors, who do not make a discernable contribution but are named in order to increase the probability of publication; and "honorary" authors, who only have a tenuous connection to the study.
Recommendations:
2.6. Before starting the research, establish the function of each researcher and the manner in which they will be recognized.
2.7. It is not necessary to mention an individual's participation in a study or publication, but if their contribution is substantial, than authorship would be justified, either as an author or assistant.
2.8. Authorship cannot be bestowed on an individual without their consent.
2.9. All of the individuals that are named as authors must meet the requirements for authorship and all of those that meet the requirements must appear as authors or assistants.
2.10. Some groups list the authors alphabetically, sometimes with a notation that indicates that all of the authors contributed equally to the study and the publication.

3. Changes in the authorship[3]

Criteria:
3.1. Additions to, removals from, and reorganization of the author names in accepted articles must be noted.
3.2. Petitions to add to, remove from, or reorganize the authors must be sent by the corresponding author of the accepted articles and must include:
a) The reason for the addition, elimination, or reorganization.
b) A written statement (e-mail) from all of the authors that confirms their agreement with the addition, elimination, or reorganization. In the case of an addition or elimination, a confirmation is also required from the author to be added or removed.

4. Conflict of interest[4]

Criteria:
4.1. When a researcher or author has a financial/personal opinion or interest that could affect their objectivity or improperly influence their actions, there exists a possible conflict of interest. Conflicts can be actual or potential.
4.2. The most evident conflicts of interest are financial, such as:
a) Direct: employment, stocks, scholarships, patents.
b) Indirect: assistantship to promoting organizations, investment funds, paid expert testimony.
4.3. Conflicts can also arise from personal relationships, academic competition, and intellectual passion. For example, an author could have:
a) Some personal interest in the results of the research.
b) Personal opinions that are in direct conflict with the research topic.
Recommendations:
4.4. Disclose all conflicts of interest, actual or potential, that inappropriately influence the findings or results of a study, including any that arise within the three (3) years after the start of said study if they could unduly (bias) influence the study.
4.5. Disclose the role of any promoter (or promoters) in the study, if any, in the design, in the collection, analysis or interpretation of the data, in the document review, or in the decision to present the document for publication.
4.6. The researchers must not enter into agreements that interfere with their access to all of the data or with their ability to independently analyze the data or to prepare and publish the manuscript.
4.7. The document must contain a statement (with the heading "Role of the financial source") in a section that is separate from the text and before the References section.
4.8. Some examples of conflicts of interest that must be revealed include: employment, consulting, stocks, honorariums, paid expert testimony, patent requests or registration, and subsidies or other financing.
4.9. All of the sources of financial support for the project must be revealed.
4.10. The role of any study sponsors must be described.

5. Duplicate publication[5]

Criteria:
5.1. Authors have the obligation of proving that their article is based on original research (never before published). The intentional submission or resubmission of a manuscript for duplicate publication is considered a breach of editorial ethics.
5.2. A duplication publication, or multiple publication, results when two or more articles, without any reference to each other, essentially share the same hypothesis, data, discussion points, and/or conclusions. This can occur to different degrees: literal duplication, partial but substantial duplication or paraphrasal duplication.
5.3. One of the main reasons that duplicate publications are considered unethical is that they can result in the "inappropriate weighting or unwitting double counting" of results from just one study, which distorts the available evidence.
Recommendations:
5.4. Articles sent for publication must be original and not sent to other editors. When sent, the authors must reveal the details of related articles (even when in another language) and similar articles being printed or translated.

5.5. Even though a submitted article is being reviewed and the final decision is not known, wait to receive notification from the editors before contacting other journals and then only do so if the editors decline to publish the article.
5.6. Avoid submitting a previously published article to another journal.
5.7. Avoid submitting articles that essentially describe the same research to more than one journal.
5.8. Always indicate previous submissions (including presentations and recorded results) that could be considered duplicate results.
5.9. Avoid writing about your research in two or more articles from different angles or on different aspects of the research without mentioning the original article.
5.10. Creating various publications based on the same research is considered a type of manipulation.
5.11. If an author wishes to send an article to a journal that is published in a different country or a different language, ask for permission from the editors first.
5.12. When submitting an article, indicate all of the details of the article that were presented in a different language along with the relevant translations.

6. Acknowledging sources

Criteria:

6.1. Authors must cite the publications that had an influence on the determination of the nature of the offered study.
6.2. Privately obtained information cannot be used without the express written consent of the source.
6.3. Republishing tables or figures requires the permission of the author or editor, who must be appropriately cited in the table or figure legend.
6.4. Information obtained through confidential services, such as arbitration articles or subsidy applications, cannot be used without the express written consent of the author of the work involved in said services.

7. Scientific fraud[6]

Criteria:

7.1. Fraud in scientific publications refers to the presentation of false data or conclusions that were not obtained through a rigorous research process.
7.2. The following types of fraud exist for the publication of research results:
a) Fabricating data. Inventing research data and results for later dissemination.
b) Falsification of data. The manipulation of research material, images, data, equipment or processes. Falsification includes the modification or omission of data or results in such a way that the research is not represented in a precise manner. A person may falsify data in order to obtain the desired final results of a study.

Recommendations:

7.3. Before submitting an article, carefully read the editorial and data policies of the journal.
7.4. Never modify, change or omit data intentionally. This includes research material, processes, equipment, tables, citations, and bibliographical references.
7.5. Fabricating and falsifying data constitute grave misconduct because both result in scientific publications that do not precisely reflect the actual observations.
7.6. Authors must appropriately manage the data that supports the research, taking special care in the compilation, production, preservation, analysis and presentation of the data.
7.7. Maintain precise records of the raw data, which must be assessable in case the editors request them after publication of the article.

8. Plagiarism[7]

Criteria:

8.1. Plagiarism is one of the more common types of misconduct in publications; it occurs when an author passes the work of others off as their own without permission, citations, or acknowledgment. Plagiarism can occur in different forms, from literally copying to paraphrasing the work of another person, including data, ideas, concepts, paragraphs, and phrases.
8.2. Plagiarism has different degrees of severity; for example:
a) The quantity of work taken from another person (various lines, paragraphs, pages, or the entire article).
b) What is copied (results, methods, or introduction section).
8.3. Plagiarism, in all of its forms, constitutes unethical behavior and is unacceptable.
8.4. Literal copying is acceptable if the source is indicated and the text is placed in quotation marks.

Recommendations:

8.5. Always remember that it is vital to recognize the work of others (including the work of your assistants or your previous studies).
8.6. Do not reproduce the work of others word for word, in totality or partially, without the permission and recognition of the original source.
8.7. Maintain a record of the sources that are used in the research and where they are used in the article.
8.8. Be sure to accurately acknowledge and cite the original source in your article.
8.9. Even when referencing the source, avoid using the work of others word for word unless it is placed in quotations.
8.10. Paraphrasing is only acceptable if the source is correctly indicated and the source's intended meaning is not changed.
8.11. Use quotations, and cite all of the content that is taken from a previously published source even when using your own words.

9. Fragmentation[8]

Criteria:

9.1.Fragmentation occurs when a large study is divided or segmented into two or more publications.
9.2. As a general rule, as long as the "fragments" of a divided study share the same hypothesis, populations, and methods, this not considered an acceptable practice.
9.3. The same "fragment" can never be published more than one time. Fragmentation can result in distortion of the literature, creating the mistaken belief in readers that the data presented in each fragment (i.e. journal article) are derived from different subject samplings. This not only distorts the "scientific database", but creates repetition that results in a loss of time for editors and evaluators that must work on each article separately. Furthermore, the cited author receives an unfair increase in their number of references.

Recommendations:

9.4. Avoid inappropriately dividing the data of one study into two or more articles.
9.5. When presenting your work, be transparent. Send copies of the manuscripts that are closely related to the manuscript in question, including published, recently submitted and accepted manuscripts.

10. Informed consent

Criteria:

10.1. Studies on patients and volunteers require the approval of the ethics committee.
10.2. The informed consent must be duly documented.
10.3. Permission and waivers must be obtained when an author wishes to include details of a case or other personal information or images of the patients or any other person.
10.4. Special care should be taken when obtaining the consent

of children (especially when a child has special needs or learning disabilities) when their head or face is displayed or when reference is made to the name of an individual or other personal data.

11. Correction of published articles[9]

Criterion:

When an author discovers a significant inexactitude or error in a published article, they must immediately notify the journal and cooperate in the correction process.

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Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». *Physics Letters B* 694, n.º 3 (noviembre de 2010): 246-51.

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[2] Elsevier, «Autoría. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

[3] William Black, Rodolfo Russo, y David Turton, «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes», *Physics Letters B* 694, n.º 3 (noviembre de 2010): 246-51.

[4] Elsevier, «Conflicto de intereses. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

[5] Elsevier, «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

[6] Elsevier, «Fraude en investigación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

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