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Effect of antibiotic residues from subclinical mastitis on β-lactoglobulin concentration in bovine and goat milk

Efecto de los residuos de antibióticos por mastitis subclínica sobre la concentración de β-lactoglobulina en leche bovina y caprina

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ABSTRACT

β-Lactoglobulin (β-LG) is an important milk protein, accounting for approximately 10% of total milk protein and 50–60% of whey proteins. Its concentration can vary depending on the species and several factors, including the use of antibiotics in the treatment of conditions such as subclinical mastitis. If withdrawal periods are not properly observed, antibiotic residues may remain in the milk, compromising its quality and posing risks to consumers. This study evaluated the effect of antibiotic residues from the treatment of subclinical mastitis on the concentration of β-LG in milk from cattle (*Bos taurus*) and goats (*Capra hircus*) on farms located in Paipa, Boyacá (Colombia). Twelve milk samples were collected from Simhol and Jerhol cattle and from Saanen and Alpina goats that tested positive for subclinical mastitis using the CMT. Samples were collected before and 24 hours after treatment with beta-lactams and tetracyclines. β-LG concentration was quantified by reverse-phase high-performance liquid chromatography (RP-HPLC), and the data were analyzed using a linear model under a 2×2 factorial design with the following factors: antibiotic residues (present–absent) and species (cow–goat). A significant reduction of 0.43 g L^{-1} (P<0.05) in β-LG concentration was observed in association with the presence of antibiotic residues, with no interaction with species. These results are exploratory and suggest a possible negative impact of antibiotics on the functional and nutritional quality of milk, potentially related to protein carbonylation processes.

Palabras clave:
Sanidad animal
Calidad alimentaria
HPLC
Proteínas de la leche
Residuos de medicamentos veterinarios

RESUMEN

La β-Lactoglobulina (β-LG) es una proteína importante de la leche, representando aproximadamente el 10% del total proteico y entre el 50-60% de las proteínas del suero. Su concentración puede variar según la especie y diversos factores, como el uso de antibióticos en el tratamiento de enfermedades como la mastitis subclínica, los cuales, si no se respetan los tiempos de retiro, pueden dejar residuos en la leche que podrían comprometer su calidad composicional e inocuidad, representando un riesgo para el consumidor final. Este estudio evaluó el efecto de los residuos de antibióticos en el tratamiento de mastitis subclínica sobre la concentración de β-LG en leche de bovinos (*Bos taurus*) y caprinos (*Capra hircus*), en fincas ubicadas en Paipa, Boyacá (Colombia). Se recolectaron 12 muestras de leche de bovinos Simhol y Jerhol y caprinos Saanen y Alpina, positivos para esta afección mediante CMT. Las muestras se tomaron antes y 24 horas después del tratamiento con betalactámicos y tetraciclinas. La β-LG se cuantificó por medio de cromatografía líquida de alta resolución en fase reversa (RP-HPLC), y los datos se analizaron mediante un modelo lineal bajo un diseño factorial 2×2 con los factores: residuos antibióticos (presencia-ausencia) y especie (vaca-cabra). Se observó una reducción significativa de 0,43 g L^{-1} (P<0,05) en la concentración de β-LG asociada a la presencia de residuos antibióticos, sin interacción con la especie. Estos resultados constituyen hallazgos exploratorios que sugieren un posible impacto negativo de los antibióticos sobre la calidad funcional y nutricional de la leche, posiblemente relacionado con procesos de carbonilación proteica.

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Milk and its derivatives from bovine and goat sources are important sources of dietary energy and essential nutrients, including calcium, magnesium, selenium, riboflavin, vitamin B12, and pantothenic acid. These nutrients contribute to strengthening the skeletal system, supporting muscle regeneration, and regulating immune function (FAO 2024). Among the main whey proteins, β-lactoglobulin (β-LG) is predominant in the milk of most mammals except guinea pigs, rabbits, rodents, and humans and has therefore been associated with allergenic potential in infants, causing symptoms that may manifest in the skin, respiratory system, and digestive tract (Giannetti et al. 2021).

β-LG is the main whey protein, representing about 10% of total milk protein and 50–60% of whey proteins. It is a globular protein from the lipocalin family, typically found in dimeric form with a molecular weight of 36 kDa and composed of 162 amino acids (Kontopidis et al. 2002). In cattle, 11 variants of β-LG have been identified, with isoforms A and B being the most common. These differ by two amino acid substitutions. Compared to bovine β-LG, caprine β-LG shows eight amino acid sequence differences, though without significant alterations to its tertiary structure (Crowther et al. 2018). It is synthesized in mammary gland epithelial cells from blood precursors and fulfills important functions, including transporting hydrophobic molecules via three binding sites, regulating phosphorus metabolism in the mammary gland, and contributing to the transfer of passive immunity to newborns (Varlamova and Zaripov 2020).

The concentration of β-LG varies by species, ranging from approximately 2–4 g L^{-1} in cattle and averaging 3.02 g L^{-1} in goats (Gołębiowski et al. 2020). Beyond its biological roles, β-LG exhibits antioxidant properties and functional characteristics valuable to agribusiness, such as gelling, foaming, emulsifying, and flavor-binding capacities (Madureira et al. 2007). However, its metabolic function can be altered by environmental conditions—mainly milk pH, protein and ion concentrations, and temperature—which may affect its chemical structure, transport capacity, and synthesis (Varlamova and Zaripov 2020). Additionally, this protein has demonstrated the ability to bind various ligands *in vitro* such as steroids, fatty acids, retinoids, vitamin D, and cholesterol, making it a potential carrier for various types of ligands, including drugs (Kontopidis et al. 2002).

Furthermore, the quality of this protein can be influenced by several factors, including the use of antibiotics to treat diseases such as mastitis (Beltrán et al. 2013). In Colombia, mastitis is estimated to cause losses of up to USD 25,000 per affected farm annually (SIC 2021). Antimicrobial drugs are used for the treatment and prevention of this condition, and they may be administered either parenterally or locally (Beltrán et al. 2013). However, irrational use poses a serious problem due to its implications for public health, milk processing, and product quality (Tarazona-Manrique et al. 2024). Among the most commonly used pharmacological groups are β-lactams and tetracyclines (Sachi et al. 2019). Studies indicate that tetracyclines bind near Trp19 within a hydrophobic domain of β-LG (Mukherjee et al. 2018), although there is no clear evidence of a specific binding site for β-lactams on this protein.

Regarding regulation, European Regulation No. 853/2004 establishes that milk intended for human consumption must not contain antibiotic residues exceeding the maximum residue limits (MRLs) set forth in Commission Regulation (EU) No. 37/2010 and the Codex Alimentarius, which includes MRLs and risk management recommendations (RGRs) for veterinary drug residues in food, as outlined in its 2023 version. This legislation sets the MRL for tetracyclines and β-lactams (cephalosporins) at 100 µg kg^{-1} (FAO and WHO 2023). In Colombia, Resolution 1382 of 2013 establishes MRLs for veterinary drugs in foods of animal origin intended for human consumption (Ministerio de Salud y Protección Social 2013).

Recent studies have shown that the presence of antibiotic residues affects both the quality and quantity of milk's nutritional components, mainly whey proteins with special emphasis on β-LG, by inducing carbonylation of certain amino acid residues, which promotes protein degradation (Marrugo-Padilla et al. 2022). Additionally, β-LG has been shown to spontaneously and moderately bind to certain veterinary drugs such as amoxicillin (Habibian-Dehkordi et al. 2022) and levamisole (Ghasemi et al. 2024), which are administered parenterally. This highlights the potential for milk contamination by these compounds, directly related to the protein's concentration, posing a public health concern.

In Colombia, no studies have been reported that evaluate the impact of antibiotic residues from the treatment of subclinical mastitis on β-lactoglobulin (β-LG) concentration

in bovine and goat milk. This gap underscores the need to further investigate interactions between milk proteins and antimicrobials commonly used in dairy production, due to their potential impact on compositional quality and product safety. Within this context, the present pilot study aimed to evaluate the effect of β-lactam and tetracycline antibiotic residues on β-LG concentration in dairy cattle milk, 24 hours after drug administration in milk of bovine and goat origin, produced on farms located in the municipality of Paipa, Department of Boyacá, Colombia.

MATERIALS AND METHODS

Study Area and Period

The study was conducted in the municipality of Paipa, Department of Boyacá (Colombia), during July 2024, which corresponds to the rainy season. The area is situated at an average altitude of 2,525 meters above sea level (masl), with an average annual temperature of 13 °C, approximately 80% relative humidity, and an annual rainfall of 944 mm. Paipa is characterized by significant agricultural activity, particularly traditional dairy farming of both cattle and goats (Acuña et al. 2022).

Animals included in the study- Experimental units

The sample size was determined based on the exploratory nature of the study and the project's budgetary constraints. Inclusion criteria included: diagnosis of subclinical mastitis at the time of sampling, absence of prior pharmacological treatments, representation of common dairy breeds in the region (Simhol and Jerhol in cattle, Alpina and Saanen in goats), and producer consent for animal participation. Additional data collected for each animal included age, lactation stage, daily milk production, and number of calvings. The study involved four cattle: two Simhol cows, aged 6 and 7 years, each with four calvings and daily milk production (DMP) of 14 L and 10 L, respectively. At the time of sampling, both cows were in the second third of lactation, and their production was evaluated based on a standardized 305-day lactation period. Also included were two Jerhol cows, aged 5 and 7 years, with three and four calvings, and DMPs of 18 L and 15 L, respectively—also in the second third of lactation. Additionally, two goats were analyzed: the first, an Alpine breed, was 3 years old, with two parturitions and a daily milk production of 3 L, in the first third of lactation (standardized at 240 days); the second, a Saanen breed, was two years old, with one parturition and a daily production of 2 L, also in the first third of lactation.

Sample collection

Animals were evaluated for the presence of mastitis using the California Mastitis Test (CMT), employing reagent provided by California Animal Health, following this protocol: the first stream of milk was discarded, and approximately 5 mL of milk was collected in the corresponding wells of the paddle. An equal volume of commercial reagent was added to each well. A gentle circular motion was applied on a horizontal plane for 20 seconds to mix the reagent with the milk. The results were collected and interpreted as follows: 0 (negative or no thickening of the sample), Trace (slight thickening that disappeared with circular motion), 1 or + (marked thickening with no tendency to form a gel), 2 or ++ (immediate thickening with slight gel formation), or 3 or +++ (gel formation with the mixture's surface raised). This evaluation reflects the inflammatory response based on the viscosity of the gel formed when the reagent interacts with the milk (Mendoza et al. 2017). The Alpine goat (individual 1) showed an initial CMT result of 2; the Saanen goat (individual 2), a result of 1; the Simhol cows (individuals 3 and 4), both had a score of 2; and the Jerhol cows (individuals 5 and 6) presented scores of 2 and 1, respectively. A first sample collection was then carried out, followed by the application of experimental treatments. Individuals 2, 3, and 5 were administered oxytetracycline 20% (tetracycline) parenterally, while individuals 1, 4, and 6 received intramammary treatment with Cobactan LC, containing 2.5% cefquinome sulfate (a β-lactam). Twenty-four hours later, a second round of sample collection was performed to assess the early-stage effect of antibiotic administration on protein concentration. The sampling procedure began with teat washing using a soap solution, followed by rinsing with sterile water and drying with sterile gauze. Teats were then disinfected with an iodine solution. Once asepsis was completed, 500 mL of milk was collected in sterilized glass jars, drawing equal amounts from the four teats in cows and two teats in goats. Samples were stored at 4 °C until they were transferred to the BIOPOLAB laboratory in Bogotá for analysis by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC). A total of twelve samples were collected and analyzed in duplicate.

Chromatographic conditions

The concentration of β-LG was determined using RP-HPLC based on a protocol developed by the BIOPOLAB laboratory, adapted from the method described by Ding et al. (2011). This methodology enabled efficient separation and accurate quantification of the protein under optimized conditions for the milk matrices analyzed. The equipment used was a Thermo Dionex Ultimate 3000 UHPLC system. Separation was performed using a ZORBAX Eclipse XDBC18 analytical column (4.6×150 mm, 5 µm film thickness). Linear gradient elution was conducted as outlined in Table 1, at a flow rate of 0.8 mL min^{-1}, using two solvents: Solvent A (acetonitrile with 0.1% trifluoroacetic acid [TFA]) and Solvent B (HPLC-grade water with 0.1% TFA). Each sample analysis lasted 42 minutes. The column was maintained at 30 °C, and detection was carried out at 220 nm using a diode array detector (DAD), with results expressed in milliabsorbance units (mAU). The injection volume was 50 µL. For calibration, a β-lactoglobulin protein standard (90% purity, Sigma-Aldrich) was used to generate a calibration curve. The resulting chromatogram displayed a stable retention time and a symmetrical peak, demonstrating the sensitivity and reliability of the method for protein quantification (Figure 1).

Table 1. Gradient elution time.

Time (min)	Phase A%	Phase B%
0	33	67
30	45	55
42	33	67

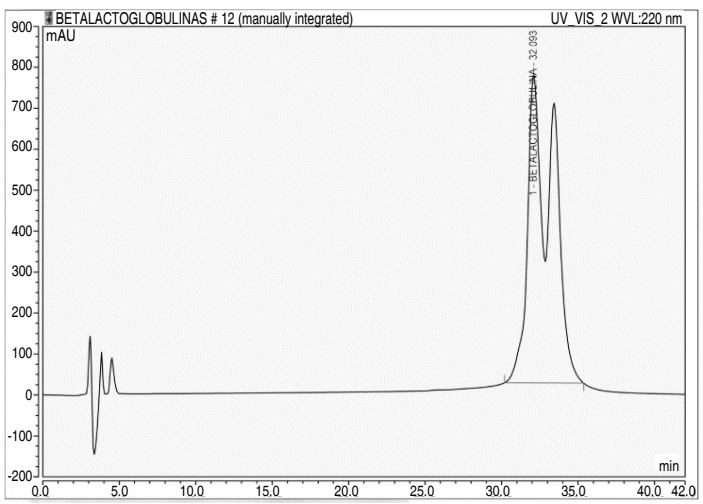

Figure 1. Chromatogram of the protein standard β-Lactoglobulin at 90% purity from Sigma-Aldrich.

Sample treatments

Two milliliters of milk sample were diluted with 14 mL of Type 1 water in a test tube. The pH of the samples was adjusted to 4.3 by adding 200 µL of acetic acid solution (10%, v/v), followed by 200 µL of 1 M sodium acetate solution. The volume was then brought to 20 mL with Type 1 water. The sample was centrifuged at 3,000 rpm and 20 °C for 10 minutes. The volume of the supernatant (SN) was recorded (approximately 18.5 mL). The resulting precipitate was acidified with 1 mM sodium acetate buffer (pH=4.3) to match the SN volume, allowed to precipitate again, and centrifuged under the same conditions. The second SN was discarded, and 620 µL of phosphate buffer was added to the resulting precipitate. The mixture was dissolved in an ultrasonic bath. The final solutions were prepared using 50 mM phosphate buffer (pH=6.8) and a mixture of Mobile Phase A and Mobile Phase B (70:30). All aliquots were filtered through a 0.22 µm nylon filter before being injected into the equipment. To bring the results within the linear working range of the calibration curve, a dilution factor of 2 was applied. From the beginning, the standards were dissolved in 50 mM phosphate buffer solution at pH=6.8. The reported values correspond to the total concentration of β-lactoglobulins in the samples,

accounting for aliquots, dilutions, and other calculations, and are expressed in g L^{-1}.

Statistical analysis

To analyze the concentration of β-LG in bovine and goat milk as a function of the presence or absence of antibiotic residues, statistical tests were conducted to verify the normality of the data, followed by fitting a one-factor variance model with two levels. First, the data distribution was evaluated using the Shapiro-Wilk normality test. Once normality was confirmed, a linear model (Equation 1) was fitted to compare the β-LG concentration between samples with and without antibiotic residues.

$$yij=\mu + \tau i + \varepsilon ij \quad (1)$$

Where yij is the concentration of β -LG, μ is the overall mean concentration of β-LG, τi is the effect of antibiotic residues, and εij is the random error of the model.

In additional analysis, a 2x2 factorial experimental design was applied to study the interaction between two factors: species (bovine or goat) and the presence and absence of antibiotic residues, as shown in Equation 2.

$$yijk = \mu + \alpha i + \beta j + (\alpha\beta)ij + \varepsilon ijk \quad (2)$$

Where $yijk$ is the concentration of β-LG for the k-th replicate of the i-th level of the species and the j-th level of antibiotic residue, μ is the overall mean β-LG concentration, αi is the main effect of the i-th level of the "species" factor (bovine or goat), βj is the main effect of the j-th level of the "antibiotic residues" factor (presence or absence), $(\alpha\beta)ij$ is the interaction effect between the i-th level of the "species" factor and the j-th level of the "antibiotic residues" factor and εijk is the random error of the model.

This design allowed the evaluation of the main effects of each factor, as well as their potential interaction on β-LG concentration. As in the previous analysis, the normality of the residuals and homoscedasticity were verified to ensure the validity of the results. Comparisons of the effects of the factors on β-LG concentration were carried out using ANOVA, with a significance level of 0.05 to determine whether significant differences existed between groups. All analyses were performed using the RStudio statistical software.

RESULTS AND DISCUSSION

Effect of antibiotic residues on β-LG protein concentration.

This study evaluated the effect of antibiotic treatments administered for subclinical mastitis on the concentration of β-lactoglobulin (β-LG) in bovine and goat milk, to identify possible alterations associated with the presence of antimicrobial residues. Table 2 presents β-LG concentration values, expressed in g L^{-1}, from milk samples analyzed in duplicate. The samples were classified by species, breed, sampling time (before and 24 h after antibiotic administration), and presence of residues. The antibiotic treatments included 20% oxytetracycline administered parenterally and cefquinome (Cobactan LC 2.5%) administered intramammarily. This dataset allows for the observation of changes in β-LG concentrations following recent antibiotic exposure and provides insights into potential differences between species and breeds. Although the sample size (n=12) is limited, the design of this pilot study using repeated measures allows for a preliminary estimation of intra-animal variability in β-LG concentration and detection of potential changes due to antibiotic administration, both parenteral and intramammary. Pilot studies of this kind are commonly employed to assess biological variability and analytical feasibility prior to the design of confirmatory studies with larger, statistically powered sample sizes (Stevenson 2021).

The concentration of β-LG protein was compared between samples with and without antibiotic residues from the β-lactam and tetracycline families. A linear model was applied using the presence or absence of antibiotic residues as a single factor. The model showed that the average β-LG protein concentration in the absence of antibiotic residues across both bovine and goat samples was 3.97 g L^{-1}. In contrast, in the presence of these antibiotic residues, the concentration decreased by an average of 0.473 g L^{-1}. The model yielded an R-squared value of 0.507, indicating that 50.79% of the variability in β-LG concentration is explained by the presence of antibiotic residues (P<0.05). This result suggests a statistically significant and substantial effect of antibiotic residues on β-LG protein concentration, as illustrated in Figure 2.

Table 2. Concentration of β-lactoglobulin (β-LG) in bovine and goat milk samples according to species, breed, sampling time, and presence of antibiotic residues.

Sample	Individual	Species	Breed	Timing (h)	Antibiotic residues	Treatment	β-LG (g L^{-1})	Duplicate (g L^{-1})
1	1	Goat	Alpine	Before	Absence	—	3.96	4.01
2	1	Goat	Alpine	24 after	Presence	Cobactan at 2.5% LC	3.69	3.67
3	2	Goat	Saanen	Before	Absence	—	3.98	3.98
4	2	Goat	Saanen	24 after	Presence	Oxytetracycline at 20%	3.73	3.72
5	3	Cow	Simhol	Before	Absence	—	3.74	3.74
6	3	Cow	Simhol	24 after	Presence	Oxytetracycline at 20%	3.12	3.13
7	4	Cow	Simhol	Before	Absence	—	3.67	3.72
8	4	Cow	Simhol	24 after	Presence	Cobactan at 2.5% LC	3.23	3.19
9	5	Cow	Jerhol	Before	Absence	—	4.27	4.25
10	5	Cow	Jerhol	24 after	Presence	Oxytetracycline at 20%	3.62	3.66
11	6	Cow	Jerhol	Before	Absence	—	4.25	4.24
12	6	Cow	Jerhol	24 after	Presence	Cobactan at 2.5% LC	3.64	3.65

Figure 2 illustrates the reduction in β-LG protein concentration in samples containing antibiotic residues. The central line for each group represents the mean β-LG concentration. In the absence of antibiotic residues, the mean concentration is approximately 4.0 g L^{-1}, with a data dispersion ranging from 3.6 to 4.2 g L^{-1}, indicating greater variability. In contrast, in the presence of antibiotic residues, the mean concentration decreases to approximately 3.5 g L^{-1}, with a narrower dispersion ranging from 3.2 to 3.7 g L^{-1}. This pattern highlights not only a reduction in protein concentration but also reduced variability in the presence of antibiotic residues.

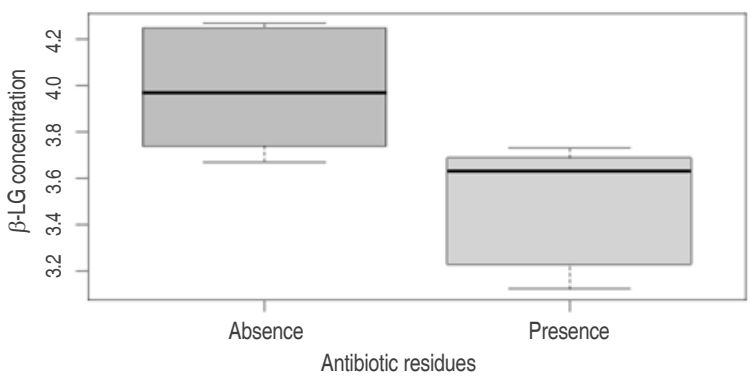

Figure 2. β-LG protein concentration as a function of the presence or absence of antibiotic residues.

Effect of the interaction between species and antibiotic residues on β-LG protein concentration

The factorial experimental design, which considered the factors of species (cow and goat) and antibiotic residue presence (present or absent), revealed that milk from bovine sources had a slightly higher β-LG protein concentration by 0.0125 g L^{-1} compared to goat milk. However, this difference was not statistically significant (P>0.05). When holding species constant, the presence of antibiotic residues resulted in a decrease of 0.26 g L^{-1} in β-LG protein concentration, regardless of the route of antibiotic administration. Regarding the interaction effect between species and antibiotic residues, a further decrease of 0.320 g L^{-1} in protein concentration was observed. However, this interaction was also not statistically significant (P>0.05), indicating that the effect of antibiotic residues on β-LG concentration does not significantly depend on the species. Overall, the model explains 60.33% of the variability in β-LG concentration, with a marginal level of significance (P=0.05), suggesting that while the model captures a substantial portion of the variation, its predictive power remains limited. These findings are illustrated in Figure 3.

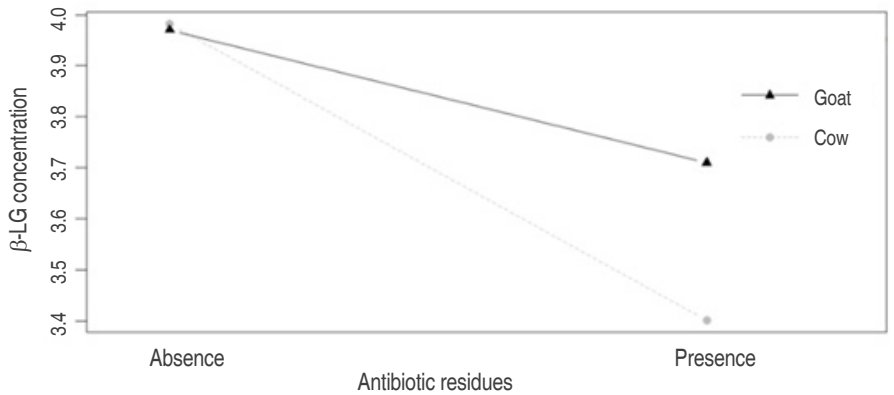

Figure 3. Protein concentration as a function of species and antibiotic residues.

These results are, to the best of current knowledge, the first of their kind reported from an investigation conducted in Colombia. Statistically, it was determined that species is not a factor influencing β-LG protein concentration in the context of subclinical mastitis, unlike the presence of antibiotic residues, which does appear to have an effect. This outcome is likely due to the reported similarity between intra- and interspecies protein isoforms (Crowther et al. 2018; Varlamova and Zaripov 2020). These findings may suggest that drugs belonging to the family of β-lactam and tetracycline antibiotics may alter β-LG protein concentration, likely due to their ability to promote carbonylation of milk proteins. This process leads to a loss of solubility and induces oxidative damage by generating stable carbonyl groups on amino acid side chains (Alviz-Amador et al. 2019), ultimately affecting β-lactoglobulin levels in both bovine and goat milk.

These effects have been previously documented. Marrugo-Padilla et al. (2020) evaluated the oxidative damage induced on caseins and whey proteins from a binary mixture of four tetracyclines and three pyrethroids. Their findings demonstrated a significant reduction in protein stability and integrity, attributed to carbonylation. Later, in an *in vitro* study, Marrugo-Padilla et al. (2022) investigated the carbonylation of caseins and whey proteins induced by tetracyclines at concentrations equivalent to maximum residue limits (MRLs). They observed irreversible carbonylation in most samples, resulting in a marked loss of solubility and a decrease in the gastric and intestinal digestibility of β-LG, thereby compromising its nutritional quality.

Similarly, Marquez et al. (2021) confirmed that both tetracycline and β-lactam residues at MRL concentrations can promote *in vitro* carbonylation in chicken breast proteins. In a follow-up study, Márquez et al. (2022) demonstrated that higher drug concentrations lead to greater oxidative effects on various meat proteins, including sarcoplasmic, myofibrillar, and insoluble proteins. These results support the hypothesis that antibiotic residues in biological matrices such as milk and meat induce

post-translational modifications in proteins via oxidative stress, with carbonylation serving as a key biomarker of this process. Importantly, these alterations not only compromise the functional properties of milk, such as solubility and digestibility, but may also have implications for human health. Recent studies have suggested that the consumption of oxidatively modified proteins is associated with the development of metabolic disorders, intestinal inflammation, and even carcinogenic processes (Muñoz et al. 2018; Estévez and Luna 2017). Consequently, adhering to antibiotic withdrawal periods is essential to allow for the gradual recovery of protein synthesis in the mammary gland, the restoration of a normal milk protein profile, and the prevention of human exposure to antibiotic residues (Mestorino et al. 2008).

Regarding pharmacokinetics, tetracyclines typically reach peak blood concentration within 6–8 hours, while β-lactams reach their maximum concentration approximately 12–18 hours after parenteral administration (Paredes 2010). This pharmacological behavior facilitates their significant excretion into milk and likely explains the observed reduction in β-LG concentration at the time of the second sampling (24 hours post-administration). Therefore, it is necessary to evaluate the effects of these drugs on β-LG concentration throughout the entire duration of their therapeutic use. Monitoring protein concentration dynamics across the complete administration and withdrawal timeline of each antibiotic will provide deeper insights into their physiological impacts on milk composition and the safety prescription period of each drug, as well as any potential residual effects that may persist days after cessation of administration.

It has been shown that β-LG concentration increases in animals suffering from mastitis of microbial origin, as β-LG possesses antimicrobial properties that may support the recovery process (Chaneton et al. 2011). Therefore, a decrease in its concentration following antibiotic administration could have implications for the animal's recovery and its ability to respond to subsequent infections that lead to mastitis.

Further research in this area is essential to translate findings into practical applications in the field. Identifying potential alterations in the concentration of biologically significant milk proteins such as β-LG within the context of drug use to treat mastitis-causing microorganisms can guide the rational use of veterinary pharmaceuticals and ensure the production of high-quality dairy products.

CONCLUSION

This study determined that the presence of antibiotic residues from the β-lactam and tetracycline families, used in the treatment of subclinical mastitis, has a statistically significant impact on the concentration of β-LG protein, reducing its average content by 0.473 g L^{-1}. No statistically significant differences were observed in β-LG concentration between the two species evaluated (cow and goat). Regardless of the specific drug administered, β-LG levels showed a significant decrease 24 hours after parenteral administration. These findings reinforce the critical importance of strictly adhering to the withdrawal periods established for each antibiotic. Compliance ensures that residue levels in milk remain below the maximum permissible limits (MRLs), in accordance with current food safety regulations. This protects public health and helps preserve the compositional and functional quality of milk.

The results presented here represent the first data of this kind reported in Colombia, underscoring the relevance and pioneering nature of this research within the national scientific landscape. To enhance the precision and reliability of future studies, it is recommended that sample sizes be increased. Furthermore, the use of advanced analytical techniques such as mass spectrometry should be implemented to enable accurate quantification of antibiotic residues in milk. Lastly, proteomic analyses, docking studies, and molecular simulations are suggested to characterize the potential conformational and functional changes of the β-LG protein in the presence of β-lactam and tetracycline residues. Such analyses may help identify critical binding regions and clarify the biochemical consequences of these drug-protein interactions.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest associated with this study.

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