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Effect of concentrate supplementation on ruminal papillae growth and rumen wall development in calves

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ABSTRACT

The growth of ruminal papillae and rumen thickness in calves subjected to two different feed rations were evaluated. Two homogeneous treatment groups were established, consisting of four individuals each (T_1 and T_2). T_1 calves received colostrum, milk, and fresh alfalfa, while T_2 calves received the same composition as the first group, supplemented with a concentrated feed. Weaning occurred at 60 days of age for both groups. After weaning, calves were nourished, and rumen wall samples were collected from four anatomical regions of the rumen from each calf. Rumen papillae dimensions were measured macroscopically, and histology was conducted using the paraffin embedding method with H&E staining. T_1 calves exhibited average values of 1.38 ± 0.250 mm for papillae length, 3.63 ± 0.245 mm for rumen wall thickness, 1.77 ± 0.312 mm for the muscular layer, and 0.5 ± 0.005 mm for the serosal layer. Conversely, T_2 animals achieved an average papillae length of 3.13 ± 0.479 mm, with a wall thickness of 7.13 ± 0.855 mm, a muscular layer thickness of 3.00 ± 0.408 mm, and a serosal layer thickness of 1.02 ± 0.019 mm. In conclusion, the feed ration with concentrate resulted in high values of ruminal papillae growth, together with a remarkable development in the serosal layer, muscular layer, and overall rumen wall in calves.

Keywords: development, feeding, feed ration, growth, histology, rumen.

RESUMEN

La presente investigación evaluó el crecimiento de las papilas ruminales y espesor del rumen en terneros sometidos a dos raciones alimenticias distintas. Se establecieron dos grupos homogéneos de tratamiento, cada uno compuesto por cuatro individuos (T_1 y T_2). Los terneros del grupo T_1 recibieron calostro, leche y alfalfa fresca, mientras que los terneros del grupo T_2 recibieron la misma composición que el primer grupo, complementada con un alimento concentrado. El destete ocurrió a los 60 días de edad para ambos grupos. Después del destete, los terneros fueron alimentados y se recolectaron muestras de la pared del rumen de cuatro regiones anatómicas diferentes de cada ternero. Las dimensiones de las papilas ruminales se midieron macroscópicamente, y se llevó a cabo una histología utilizando el método de inclusión en parafina con tinción H&E. Los terneros del grupo T_1 mostraron valores promedio de $1,38 \pm 0,250$ mm para la longitud de la papila, $3,63 \pm 0,245$ mm para el grosor de la pared del rumen, $1,77 \pm 0,312$ mm para la capa muscular y $0,5 \pm 0,005$ mm para la capa serosa. En cambio, los animales del grupo T_2 alcanzaron una longitud promedio de papila de $3,13 \pm 0,479$ mm, con un grosor de pared de $7,13 \pm 0,855$ mm, un grosor de capa muscular de $3,00 \pm 0,408$ mm y un grosor de capa serosa de $1,02 \pm 0,019$ mm. En conclusión, la ración alimenticia suplementada con concentrado resultó en valores elevados de crecimiento de las papilas ruminales, junto con un notable desarrollo en la capa serosa, la capa muscular y en general en la pared del rumen en terneros.

Palabras clave: alimentación, crecimiento, desarrollo, histología, ración alimenticia, rumen.

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INTRODUCTION

In all beef and dairy herds, the health, growth, and productivity significantly hinge upon nutritional and feeding management practices (Parsons and Allison, 1991). Each calf, from its inception, embodies an opportunity to either sustain or augment the herd size, enhance herd genetics, or augment financial returns. The objective of rearing calves from birth to weaning is to optimize growth and mitigate health issues. An imperative facet is an understanding of the digestive system, along with an good knowledge of feeding alternatives that can meet their nutritional needs and encourage the development of their digestive physiology.

At birth, calves are considered to be physically and functionally distinct from adult animals, particularly concerning their gastrointestinal system. Although anatomical characteristics distinguishing a ruminant from a monogastric organism are present, the reticulum, rumen, and omasum exist in an underdeveloped state. Simultaneously, esophageal drip is evident, and the abomasum exhibits a developed enzymatic state. Consequently, the newborn ruminant operates as a monogastric, relying on milk-based rations until the initiation of dry matter intake propels growth and facilitates the physical and metabolic maturation of the rumen. This developmental shift enables the utilization of fibrous feeds (Van Ackeren *et al.*, 2009; Wickramasinghe *et al.*, 2019). The commencement of dry matter intake in young ruminants is imperative from the first week of age, given that the transition from a pre-ruminant to a fully functional ruminant holds vital significance (Coverdale *et al.*, 2004).

The primary objective in early calf feeding is to attain the optimal development of rumen papillae, as the morphological characteristics of these papillae play a pivotal role in digestive physiology by augmenting the surface area for nutrient absorption (Dieho *et al.*, 2016). Consequently, the transition from a liquid to a solid diet, along with the choice of feed, serves to stimulate or expedite the development of the gastrointestinal tract, particularly the rumen, inducing a series of anatomical and physiological alterations (Suarez *et al.*, 2007; Van Niekerk *et al.*, 2021). Thus, meticulous care and management pre-weaning assume paramount significance. Failure to achieve adequate rumen development and dry matter intake at the weaning stage can impede subsequent growth trajectories (Van Ackeren *et al.*, 2009). Cattle exhibiting enhanced efficiency display a thicker rumen epithelium in comparison to their less efficient counterparts (Lam *et al.*, 2018).

Hence, contingent upon the quantity and composition of the feed supplied (whether forage or a balanced diet), the fermentation of soluble carbohydrates by the rumen microbiota is poised to induce the synthesis of volatile fatty acids, with particular emphasis on butyric and propionic acids. This process, in turn, facilitates the advancement of the epithelium, synergistically supported by the development of musculature, peristaltic movements, and an increase in rumen volume (Nemati *et al.*, 2015). Additionally, the inclusion of probiotics can contribute to fostering optimal rumen health, thereby promoting superior bodily growth outcomes (Hu *et al.*, 2019).

In order to enhance feed utilization and optimization, it is necessary to quantify and scrutinize the impacts of various rations on the early maturation of rumen papillae in ruminants across diverse altitudinal levels. Consequently, the objective of this study was to assess histologically the growth and development of rumen papillae in Holstein heifers subjected to

two nutritional rations under the conditions prevalent in the highlands of Peru.

MATERIALS AND METHODS

Location

Holstein calves were reared on two farms located in the district of Cajamarca, at an altitude of 2749.53 meters above the sea level. The region has an average annual temperature of 14.62 °C, an annual relative humidity of 70.94%, and an annual rainfall of 416.6 mm (Nasa Power, 2019). Rumen histological samples were obtained during processing at the Instituto Nacional de Innovación Agraria INIA – Los Baños del Inca - Cajamarca. The staining of these histological samples took place at the SENASA Lima Laboratory, while the subsequent histological analysis occurred at the Laboratorio de Embriología e Histología of the Facultad de Ciencias Veterinarias from the Universidad Nacional de Cajamarca.

Experimental design

Eight neonatal Holstein calves were selected from two barns, each of which adopted comparable management systems and housed four calves. The average initial live weight of the selected calves was 37.63 ± 2.07 kg. Two distinct experimental groups were established, following traditional management protocols. The first group ($T_1 = 04$) received a diet comprising colostrum, milk and fresh alfalfa. In contrast, the second group ($T_2 = 04$) received a more comprehensive diet consisting of colostrum, milk, fresh alfalfa, and a commercial concentrate (CP 18%, CF 10%, fat 2.5%, moisture 14%, ash 8%, monensin 85 mg/kg).

Weaning was uniformly implemented for both cohorts at 60 days of age. Clean, fresh water was continuously fed to the calves throughout the experimental period. The quantities of feed allocated per calf over the course of the study are presented in table 1.

Only two variables were selected for analysis: the length of ruminal papillae and the thickness of the rumen wall structures. This choice is grounded in the recognition that the overall increase in papillae dimensions, both in length and width, directly impacts nutrient absorption. In other words, as the surface area of the papillae increases, the absorption rate is enhanced. This phenomenon is explained by the relationship between the number of cells, the size of the villi, and, consequently, the total area of nutrient absorption (Maiorka, 2004; Bannink *et al.*, 2008; Malhi *et al.*, 2013).

Sample collection

At the conclusion of the weaning process, calves were processed at the Camal Municipal de Cajamarca under the supervision of trained personnel. During *postmortem* examination, tissue samples (1 cm²) were collected from four anatomical regions of the rumen, specifically the dorsal rumen sac, ventral rumen sac, dorsal caudal blind sac, and ventral caudal blind sac. Subsequently, in the laboratory, the size of ruminal papillae and the thickness of tissue layers were measured using a millimeter ruler.

Next, the samples were processed using the Paraffin Embedding method. The samples were fixed in 10% buffered formaldehyde, dehydrated with increasing concentrations of ethyl alcohol (80°, 95°, and 100°). Clearing was performed with xylene in Coplin jars, followed by impregnation and embedding in paraffin.

Day	Group T ₁			Group T ₂			
	Colostrum (L)	Milk (L)	Green alfalfa (kg)	Colostrum (L)	Milk (L)	Green alfalfa (kg)	Compound feed (g)
01 - 04	4			4			
05 - 07		4			4		
08 - 14		4			4		100
15 - 21		4			4		150
22 - 28		5			5		200
29 - 35		5			5		250
36 - 42		4			4		350
43 - 49		4	3		4	3	500
50 - 56		3	5		3	5	750
57 - 63		2	7		2	7	1000

Table 1. Feeding regimen applied to each calf within the two groups during the study period.

Group T₁ calves (n = 4) were provided with a diet comprising colostrum, milk, and fresh alfalfa. On the other hand, Group T₂ calves (n = 4) received a diet consisting of colostrum, milk, fresh alfalfa, and a concentrate feed (CP 18%, CF 10%, fat 2.5%, moisture 14%, ash 8%, monensin 85 mg/kg). Weaning for both groups occurred at 60 days.

Rumen		Anatomical region				Mean ± DS (mm)	Group
		Dorsal sac	Ventral sac	Blind sac			
Parameter	Structure			Caudodorsal	Caudoventral		
Length	Ruminal papilla	1.00	1.50	1.50	1.50	1.38 ± 0.250	T ₁
		2.50	3.50	3.00	3.50	3.13 ± 0.479	T ₂
Thickness	Wall	3.51	3.51	3.51	4.00	3.63 ± 0.245	T ₁
		6.00	8.00	7.00	7.51	7.13 ± 0.855	T ₂
	Muscular	2.06	1.50	1.50	2.02	1.77 ± 0.312	T ₁
		2.50	3.00	3.00	3.50	3.00 ± 0.408	T ₂
	Serosus	0.50	0.50	0.51	0.50	0.50 ± 0.005	T ₁
		1.00	1.00	1.04	1.02	1.02 ± 0.019	T ₂

Table 2. Measurements of ruminal papillae and thickness (wall, muscular layer, and serosa) in the four anatomical regions of the rumen in Holstein calves in both groups.

Statistical difference between T1 and T2 (Student's t-test, p < 0.01). The calves in T1 (n = 4) were exclusively fed with colostrum, milk, and fresh alfalfa. The calves in T2 (n = 4) received the same diet, along with a concentrated feed (CP 18%, CF 10%, fat 2.5%, moisture 14%, ash 8%, monensin 85 mg/kg).

SD: Standard deviation.

The paraffin blocks were sent to the SENASA Laboratory in Lima. Then microtomy was performed, obtaining cuts with a thickness that ranged between 5 and 8 µm. Subsequently, they were mounted with Canada balsam and stained with Hematoxylin-Eosin. From the cuts made for each sample, three with the highest resolution were selected through microscopic observation at 100X magnification under an optical microscope.

Statistical analysis

The averages and standard deviations of rumen papillae size measurements and rumen tissue thickness were calculated. The Student's t-test was employed to identify differences between the averages of groups T₁ and T₂. A significance level of p < 0.05 was considered. The analyses were conducted using SPSS software version 27.0.1.

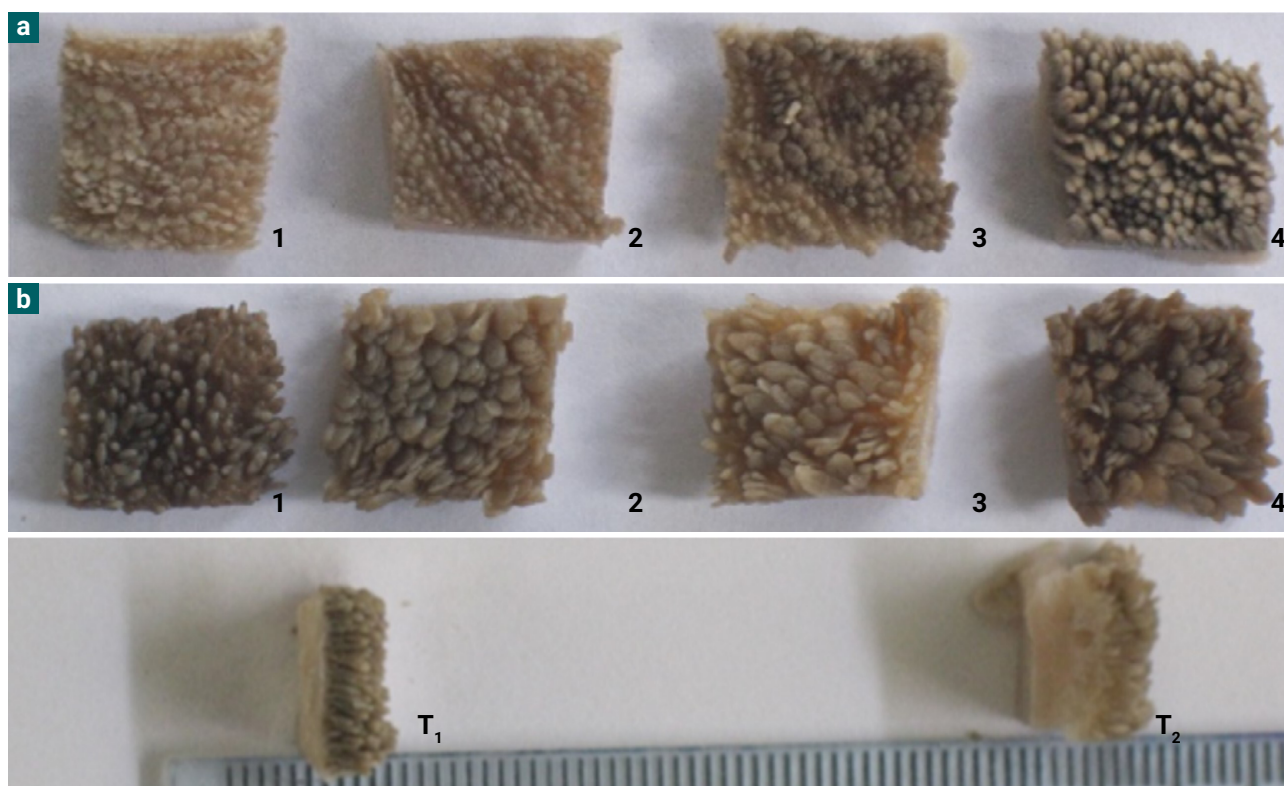


Figure 1. Presentation of rumen papillae in 1 cm² in different regions of the rumen at T₁ (A) and T₂ (B): 1. Dorsal rumen sac, 2. Ventral rumen sac, 3. Caudo-dorsal blind sac, 4. Caudo-ventral blind sac.

The papillae and the thickness of the rumen structures in T₂ exhibit greater robustness. The calves in T₁ (n = 4) were exclusively fed with colostrum, milk, and fresh alfalfa. The calves in T₂ (n = 4) received the same diet, along with a concentrated feed (CP 18%, CF 10%, fat 2.5%, moisture 14%, ash 8%, monensin 85 mg/kg).

RESULTS

Calves from T₂, supplemented with concentrated feed exhibited higher values in ruminal papillae measurements and thickness of rumen wall sections (wall, muscular, and serosal) compared to measurements of calves from T₁ that were not supplemented with concentrated feed (table 2, figures 1 and 2).

DISCUSSION

The measurements in calves supplemented with concentrate reveal robust ruminal development, with a wall thickness of 7.13 ± 0.855 mm and papillary height of 3.00 ± 0.408 mm. These values surpass those reported in a comparable study in which, among four concentrate-fed treatment groups subjected to various processes and evaluated at 8 weeks of age, the highest values were observed in calves fed with meal. In that study, the rumen papillae had a height (length) of 1.93 mm, and rumen wall thickness measured 2.62 mm. The treatments ranked in decreasing order of performance were meal + fodder, pelleted, and extruded (Castro-Flores and Elizondo-Salazar, 2012).

In another study that reported values lower than those in the current work, calves fed with corn silage *ad libitum*, concentrate, water, and milk replacer comprised the best-performing group ($p < 0.05$). This group exhibited low milk intake and high concentrate consumption. In the ruminal atrium, the ruminal papillae measured 1.89 ± 0.14 mm in length and 0.81 ± 0.06 mm in

width. In the ventral rumen sac, the papillae had lengths and widths of 1.25 ± 0.08 and 0.54 ± 0.06 mm, respectively. Finally, in the ventral blind rumen sac, the papillae measured 0.67 ± 0.08 mm in length and 0.40 ± 0.07 mm in width. Additionally, the study observed an increasing trend in plasma IGF-1 concentration in early-weaned calves ($p = 0.1$), positively correlated with ruminal papillae length ($p < 0.10$) (Zitnan *et al.*, 2005). It is worth noting that the evaluation in that study was conducted on calves at 41-day-old calves, unlike the present study which was carried out at 60 days of age.

Similarly, highly concentrated diets demonstrated a notable enhancement in the development of rumen papillae in Korean beef cattle. Across all treatment groups, significant increases in lengths, widths, mucosal thickness, and rumen wall thickness of papillae located in nine rumen locations were observed compared to the control ($p < 0.05$) (Reddy *et al.*, 2017), with values closely aligning with those found in the present study.

While forage has been shown to cause papillae shortening (Castells *et al.*, 2013), supplementation with oat hay in pre-weaning calf diets improves rumen productivity and development. This improvement is manifested in increased overall dry matter intake and rumination, reduced non-nutritive oral behaviors, improved rumen pH, and ensured healthy rumen development (Lin *et al.*, 2018). Additionally, despite higher intake, the inclusion of alfalfa hay in starter diets positively influences calf growth performance (Beiranvand *et al.*, 2014).

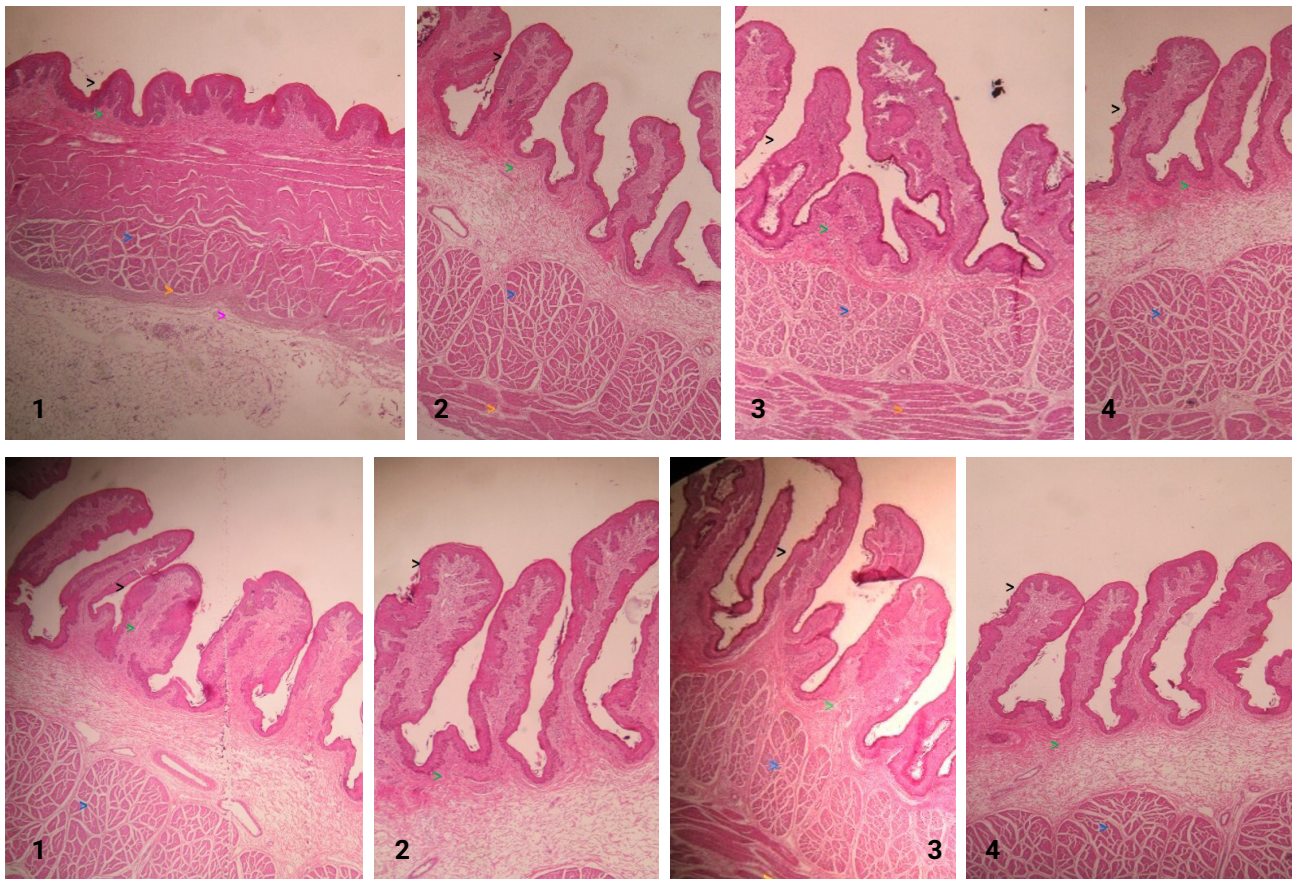


Figure 2. Histological findings of the rumen papillae in the different regions of the rumen according to T₁ (Upper) and T₂ (Lower). 1. Dorsal rumen sac, 2. Ventral rumen sac, 3. Caudo-dorsal blind sac, 4. Caudo-ventral blind sac., a. Ruminal papillae lined with a flat stratified epithelium of a bright pink color (>), b. Cell layer underlying the areolar connective tissue lining epithelium (>), c. Internal circular muscular layer (>), d. Internal circular muscular layer (>), e. Serous layer (>). (Hematoxylin-Eosin stain. 100X). The calves in T1 (n = 4) were exclusively fed with colostrum, milk, and fresh alfalfa. The calves in T2 (n = 4) received the same diet, along with a concentrated feed (CP 18%, CF 10%, fat 2.5%, moisture 14%, ash 8%, monensin 85 mg/kg).

In the present study, the inclusion of fresh alfalfa should not have significantly influenced the results, as it was fed equally to both groups per animal. A separate study on Jersey steers indicated no significant changes in length and width of ruminal papillae between treatments that included both forage and concentrate at varying concentrations (Novak *et al.*, 2019).

The introduction of solid feed at an early age is crucial, as rumen papillae respond to changes in fermentable organic matter intake, and the magnitude of the response depending on the rate of feed intake increase. This response in the surface area of ruminal papillae facilitates the uptake of volatile fatty acids (Dieho *et al.*, 2016). However, an excess of highly fermentable feed or feed restriction can alter the stratified squamous rumen epithelium, leading to damage due to high microbial fermentation activity, decreased pH (acidity), high osmolarity, toxins, and immune mediators. This may even trigger systemic inflammation. Excessive butyrate, used to support ruminal barrier development in calves at weaning, can result in hyperkeratosis, parakeratosis, and epithelial damage in the fully developed rumen of adult cows (Aschenbach *et al.*, 2019).

Papillae growth or length, essential for ruminal development, is influenced by diet (Lesmeister *et al.*, 2004). Increased length of ruminal papillae is age-related, linked to chemical stimula-

tion by volatile fatty acid concentrations (Khan *et al.*, 2008), and the presence of butyrate from fermentative digestion of carbohydrates (Klein *et al.*, 1987). The physical form of the solid feed affects papillae size, impacting the concentration of cellulolytic or amylolytic bacteria (Beharka *et al.*, 1998). Similar to our study, Sato *et al.* (2010), reported greater amylolytic bacteria and VFA production resulting in increased growth of ruminal papillae in calves where concentrate was added to their diet based on high starch feed.

Beyond the effect of butyrate (Kristensen *et al.*, 2007), other factors influencing rumen development in calves include endocrine changes promoted by colostrum growth factors (Elizondo-Salazar, 2007). However, in the present research, both treatments received the same base diet, differing only in the presence/absence of a balanced concentrate feed. Colostrum and milk were administered to both groups, eliminating the expectation of differential effects attributable to these factors. Main effects resulting from the addition of a balanced feed, promoting rumen fermentation and development, align with previous reports (Laarman and Oba, 2011; Elizondo-Salazar and Sánchez-Álvarez, 2012; Schäff *et al.*, 2018).

Prior research has emphasized the significance of starter diets in achieving favorable growth rates by improving rumen

papillae development in young calves at an early age (Bach *et al.*, 2007). An effective strategy for this purpose involves incorporating a high-energy concentrate feed that provides a greater opportunity for calves to increase digestible dry matter intake, achieve high average daily gain, and produce volatile fatty acids at an accelerated rate (Suarez *et al.*, 2007).

CONCLUSIONS

The study concludes and confirms that the inclusion of balanced feed into the diets of calves raised in Peruvian highland conditions results in the development of larger ruminal papillae. In addition, an increase in the thickness of the rumen wall, muscle and serosa is observed. These findings suggest potential implications for improved nutritional performance.

DECLARATIONS

Ethical considerations

All procedures were in accordance with European ethical regulations and the Ley de Protección y Bienestar Animal del Perú (European Directive 2010/63/EU and Ley N° 30407; respectively) on the use of animals in scientific research. The animals were processed in accordance with the Reglamento Sanitario del Faenado de Animales de Abasto del Perú (D.S. N° 015-2012-AG).

Credit author statement

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Data availability

All data pertinent to this study are included in this study.

Author contributions

EB and FC conceptualized, designed the methodology, supervised, and conducted the research. TT and LVR contributed to the Software, validation, data curation, writing-preparation of original drafts, and also collaborated in viewing, writing-reviewing and editing the manuscript. All authors approved the final manuscript.

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