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Original

Protein fragment intake and digestibility of protein in diets supplied to Chino Santandereano cattle

David Contreras-Marquez[1*] Ph.D; Juan Quintero-Pardo[1] Zoot; Edwin Correa-Rojas[1] M.Sc; Emiro Canchila-Asencio[2] Ph.D; Heidy Gutiérrez-Zocadagui[1] Zoot.

[1]Universidad de Cundinamarca, Facultad de ciencias agropecuarias. Programa de zootecnia. Laboratorio de nutrición animal, grupo de investigación GIFBA, Fusagasugá, Colombia.
[2]Instituto Universitario de la Paz, Facultad de Medicina Veterinaria y Zootecnia. Grupo de investigación CIDICA, Barrancabermeja, Colombia.
*Correspondencia: davidestebas@hotmail.com

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ABSTRACT

Objective. To quantify the concentration and consumption of the protein fragments (NPN, TP, RDTP, SDTP, NDIP, and ADIP) present in the diet supplied to cattle, and its effect on the digestibility of crude protein. **Materials and methods.** Diets offered to Chino Santandereano cattle in stables, receiving different levels of supplementation, were analyzed. Using four animals in the 4x4 Latin square design, the treatments were UNS, not supplemented; LOW, supplemented with an amount relative to 0.5% of body weight; MEDIUM, supplemented with an amount relative to 1.0% of body weight; HIGH, supplemented with an amount relative to 1.5% of body weight. Consumption was determined daily, and digestibility through total stool collection in the last two days of each period. **Results.** A higher concentration of crude protein was found in the supplement than in grass ($p<0.001$), the forage exhibiting a higher concentration of CPNPN ($p<0.001$). The supplement also presented a higher concentration of TP ($p<0.001$) and RDTP ($p=0.027$). Supplemented animals presented higher consumption of CP, CPNPN ($p=0.037$), TP, NDIP, RDTP, ADIP, and SDTP ($p<0.05$), however, when the concentration representing the consumption of ADIP in the consumption of CP was determined, no difference was observed between supplemented and UNS ($p=0.078$). Higher digestibility of CP was found in supplemented animals than in UNS ($p<0.001$), and an upward linear effect was observed between supplemented treatments as the level of supplementation increased. **Conclusions.** Supplementation improves the digestibility of crude protein by providing a greater amount of highly digestible nitrogenous fragments.

Keywords: Animal nutrition; fiber; nitrogen; protein supplements; ruminants (*Sources: CAB*).

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RESUMEN

Objetivo. Cuantificar la concentración y consumo de los fragmentos de la proteína (NNP, PV, PVAD, PVLD, PIDN y PIDA) presente en la dieta suministrada a bovinos y su efecto en la digestibilidad de la proteína bruta. **Materiales y métodos**. Fueron analizadas dietas ofrecidas a bovinos Chino Santandereano en estabulación, recibiendo diferentes niveles de suplementación, utilizando cuatro animales en delineamiento en cuadrado latino 4x4, siendo los tratamientos: NS, no suplementados; BAJO, suplementados con cantidad relativa al 0.5% del peso corporal; MEDIO, suplementados con cantidad relativa al 1.0% del peso corporal; ALTO, suplementados con cantidad relativa al 1.5% del peso corporal. El consumo fue determinado diariamente y la digestibilidad a través de colecta total de heces en los dos últimos días de cada periodo. **Resultados**. Mayor concentración de proteína bruta fue encontrada en suplemento en comparación con el pasto ($p<0.001$), exhibiendo el forraje mayor concentración de PBNNP ($p<0.001$), a la vez que el suplemento presentó concentración más elevada de PV ($p<0.001$) y PVAD ($p=0.027$). Animales suplementados presentaron mayor consumo de PB, PBNNP ($p=0.037$), PV, PIDN, PVAD, PIDA y PVLD ($p<0.05$), no obstante, cuando se determinó la concentración que representa el consumo de PIDA en el consumo de PB, no se observó diferencia entre suplementados y NS ($p=0.078$). Mayor digestibilidad de la PB fue encontrada en animales suplementados cuando contrastados con NS ($p<0.001$), observándose entre tratamientos suplementados un efecto lineal ascendente a medida que aumentó el nivel de suplementación. **Conclusiones**. La suplementación mejora la digestibilidad de la proteína bruta por aportar mayor cantidad de fragmentos nitrogenados de alta digestibilidad.

Palabras clave: Fibra; nitrógeno; nutrición animal; suplementos de proteína; rumiantes (*Fuentes: CAB*).

INTRODUCTION

Proteins are one of the most important macronutrients in animal metabolism, forming part of the structure of organs, tissues, and enzymes and are a source of nitrogen for ruminal microorganisms. They must be present in the diet in amounts that meet the animal's needs.

Protein requirements are typically calculated based on crude (CP), metabolizable (MP), and net (NP) protein. In this sense, using the crude protein requirement as the nutritional goal could generate unexpected productivity, since the crude protein cannot generate the necessary quantity of metabolizable protein, given the potential variability in the quality of the offered protein and the associated degree of digestibility of each of its components (1).

The nitrogen present in proteins has been classified according to the degree of availability in fractions A, B1, B2, B3, and C (2). "A" represents non-protein nitrogen (NPN), a highly degradable fraction at ruminal level; "C" represents the unavailable fraction of the crude protein attached to the fiber that is insoluble in acid detergent (ADF) and is thereby denoted as protein insoluble in acid detergent (ADIP). Fraction "B" represents a large part of the true protein (TP), determined by subtracting fraction A from CP. Fraction "B3" represents the true slow degradability protein (TPSD), determined by the difference between the protein adhered to neutral detergent insoluble fiber (NDF), called neutral detergent insoluble protein (NDIP), and ADIP. The fraction "B1" represents the highly digestible TP (TPHD) determined by subtracting NDIP from TP. The fraction "B2" represents the protein with an intermediate degradation rate, corresponding to the remaining nitrogen (3).

Raw materials used in animal feed contains high concentrations of indigestible or slowly degradable fractions and could compromise the contribution of metabolizable protein and the productive performance of ruminant animals (4).

Grass-raised cattle usually have limited protein intake (1), due to its low concentration in most tropical grasses. In addition, the protein in the forage could present a high concentration of slowly degrading and indigestible nitrogen fractions, given the high proportion of NDF and ADF in the dry matter of pastures (5,6). Therefore, supplementation with raw materials with a high protein and energy concentration and a low fiber concentration could improve protein digestibility, under the assumption the supplement would contain a lower concentration of nitrogenous fractions adhered to fiber (7).

Chino Santandereano cattle may not be able to express the full productive potential stipulated by genetics if they cannot maximize the degradation of the nutritional components present in ingested pastures. According to Lazzarini (8), supplementation could increase the use of nutritional components and productive performance.

Therefore, this study was conducted to quantify the consumption of the crude protein fragments (NPN, TP, TPHD, TPSD, NDIP, and ADIP) present in the diet supplied to Chino Santanderean cattle, and its effect on digestibility of crude protein.

MATERIALS AND METHODS

Location, experimental outline, and diets. The experiment was conducted at the Santa Lucia agrarian station of the University Institute of La Paz, Barrancabermeja, Santander, Colombia.

Four animals of the Chino Santandereano breed were used in a 4×4 balanced Latin square design, assigned to one of four treatments: UNS, unsupplemented; LOW, supplemented with an amount relative to 0.5% of body weight (BW); MEDIUM, supplemented with an amount relative to 1.0% of the BW; HIGH, supplemented with an amount relative to 1.5% of the BW. The supplement offered was composed of soybean meal (34.34%), palm kernel cake (10%), ground corn kernel (27.33%), and rice bran (27.33%), formulated to provide 25% of CP based on the dry material. In addition to the supplement, all animals, regardless of the treatment applied, received hay from *Brachiaria humidicola* (Rendle) Schweick. (Poaceae) and mineralized salt *ad libitum*.

During the investigation, the animals were kept in individual 3 m^2 bales, with a covered ceiling and provision of salting feeders, drinking troughs, and meshes for the controlled supply of forage. Hay and supplement were offered daily in two servings, always at 07:00 and 15:00.

Experimental procedures. The investigation lasted 115 days, corresponding to four periods of 25 days each, plus five days between the experimental periods to reduce the residual effects of the applied treatments. Individuals were rotated between the treatments at the end of each period. The first 22 days of each experimental period were used to adapt the animals to the total diet, and the remaining three days were used to collect samples of hay, supplement, and feces, which were sent to the animal nutrition laboratory at the University of Cundinamarca for chemical analysis.

Hay and supplement consumption were quantified daily during the 25 days of each period, by the difference between the quantity offered and the leftovers present in the feeders 24 hours after the offer of each of the foods. Protein consumption was estimated using the following equation (E1) (9):

$$CPI=([IDMH \times \%CPH] + [IDMS \times \%CPS]) \quad (E1)$$

in which;
IDMH is the consumption of dry matter from the basal diet;
%CPH is the crude protein concentration in the basal diet;
IDMS is the supplement dry matter consumption;
%CPS is the crude protein concentration in the supplement.

Consumption of each crude protein fragment was estimated using the following equation (E2) (9):

$$IY = ([ICPH \times \%YH] + [ICPS \times \%YS]) \quad (E2)$$

in which;
IY is the protein fragment consumption (TP, NPN, TPHD, NDIP, ADIP, and TPSD);
CPBH is the consumption of crude protein from the basal diet;
%YH is the concentration of the fragment in the crude protein of the basal diet;
CPBS is the crude supplement protein consumption;
%YS is the concentration of the fragment in the crude supplement protein.

All feces were collected from the concrete floor immediately after defecation to quantify the excretion of feces during the last two days of each experimental period. Excretion was the average amount of feces during the two days of collection. Protein excretion was estimated using the following equation (E3) (9):

$$CPE = (CDME \times \%CPF) \quad (E3)$$

in which;
CPE is the crude protein excretion;
CDME is the amount of dry matter excreted and
%CPF is the crude protein concentration in the feces.

Crude protein digestibility was calculated using the following equation (E4) (9):

$$CPD = ([CPI - CPE]/ CPI) \times 100 \quad (E4)$$

Chemical analysis. Hay, supplement, and feces samples were analyzed to determine concentrations of dry matter (DM) (INCT-CA G-003/1) and crude protein (CP) (INCT-CA N-001/1). Hay and supplement samples were also analyzed to quantify the CP concentration of non-protein nitrogen (NPN) and true protein (TP) (INCT-CA N-002/1), neutral detergent insoluble protein (NDIP) (INCT -CA N-004/1) following analysis of neutral detergent insoluble fiber (NDF) (INCT-CA F-002/1), and acid detergent insoluble protein (ADIP) (INCT-CA N-005/1) following analysis of acid-insoluble fiber (ADF) (INCT-CA F-004/1) (10).

Highly digestible true protein (TPHD) was determined using the following equation (E5) (9):

$$TPHD=(CP - [CPNPN + NDIP]) \text{ or } (TP–NDIP) \quad (E5)$$

in which
CPNPN is crude protein originating from non-protein nitrogen.

TPSD was determined using the following equation (E6) (9).

$$TPSD= (NDIP - ADIP) \quad (E6)$$

Statistical analysis. For all statistical procedures, R software (version 3.6.1) was used, and significance was assigned at $p<0.05$. All the results obtained from the variables studied were subjected to Kruskal-Wallis ANOVA to check for normality and Levene's for homogeneity of variances. Subsequently, the sum of squares was decomposed using orthogonal contrasts, constructed to evaluate the effects of supplementation, and the linear and quadratic effects of the levels of supplement (0.5, 1.0, and 1.5% of the CP). For variables that had no effects between supplemented treatments and UNS, but a significant linear or quadratic effect, a Dunnett's test was performed to identify whether a supplemented treatment differed from UNS treatment.

RESULTS

Protein fractionation present in hay and supplement. Hay had a higher concentration of NDF and ADF than the supplement ($p<0.001$). A higher concentration of crude protein ($p<0.001$), TP ($p<0.001$), and TPHD ($p=0.027$) were found in the supplement compared to hay. However, hay had a higher concentration of CPNPN ($p<0.001$), with no differences between the two raw materials in terms of the concentrations of NDIP ($p=0.544$), ADIP ($p=0.118$) and TPSD ($p=0.220$) (Table 1).

Table 1. Composition of the crude protein present in hay and supplement offered to bovine Chino Santandereano in stables.

Item	HAY				SEM	Average	Supplement				SEM	Average	p-value
	P1	P2	P3	P4			P1	P2	P3	P4			
NDF, %DM	85.90	84.26	84.27	85.42	0.990	84.962[a]	49.09	53.61	55.23	51.37	0.990	52.331[b]	<0.001
ADF, %DM	83.06	84.16	83.06	84.90	0.992	83.795[a]	42.55	48.10	48.09	45.27	0.992	46.004[b]	<0.001
CP, %DM	5.74	5.73	5.77	6.02	0.067	5.815[b]	22.64	26.40	27.32	25.35	1.013	25.429[a]	<0.001
CPNPN, %CP	24.86	29.04	29.07	24.42	1.277	26.846[a]	6.95	2.55	3.99	14.48	2.658	6.996[b]	<0.001
TP, %CP	75.15	70.96	70.93	75.57	1.277	73.154[b]	93.05	97.45	96.01	85.52	2.658	93.004[a]	<0.001
NDIP, %CP	69.17	57.42	61.66	62.19	2.434	62.608	68.54	69.22	63.41	58.32	2.539	64.869	0.544
TPHD, %CP	5.97	13.55	9.27	13.39	3.998	10.546[b]	24.51	28.23	32.60	27.20	1.681	28.135[a]	0.027
ADIP, %CP	50.33	52.98	50.52	54.50	1.008	52.082	50.72	38.49	47.60	49.84	2.802	46.666	0.118
TPSD, %CP	18.84	4.44	11.14	7.69	3.942	10.527	17.82	30.73	15.57	8.48	3.942	18.150	0.220
ADIP, %NDIP	72.76	92.28	81.93	87.63	4.201	83.652	74.01	55.61	75.08	85.47	6,206	72.542	0.188

CP: crude protein; CPNPN: protein originating from non-protein nitrogen sources; TP: true protein; NDIP: protein insoluble in neutral detergent; TPHD: highly available true protein; ADIP: protein insoluble in acid detergent
[a, b, c] different letters denote statistical difference $p<0.05$ between hay and supplement.

When the concentration that represents the ADIP within the NDIP was determined, no difference (p=0.188) between hay and supplement was also observed (Table 1).

Consumption of medium and high digestibility nitrogenous fractions. For the variables consumption of CP, CPNPN, TP, and TPHD from hay, no difference was observed between supplemented and non-supplemented animals; however, a decreasing linear effect was observed for CP and TP of hay as the level of supplementation increased (p<0.05) (Table 2).

Higher consumption of CP, CPNPN, TP, and TPHD of the supplement and total diet was observed in supplemented than non-supplemented animals (p<0.05), and as the level of supplementation increased, we observed an ascending linear effect for the consumption of CP, TP, and TPHD of the supplement and total diet (p<0.001) (Table 2).

Table 2. Consumption of medium and high availability fragments, which are part of the crude protein present in hay and supplement offered to Chino Santandereano cattle in stables.

Item	Treatments				SEM	p-value, Contrasts		
	UNS	Low	Medium	High		S vs. UNS	L	Q
CP-Hay, g	342.97	362.32	323.13	283.50	34.49	0.491	0.011	0.992
CP-Supplement, g	0.00	360.79	704.14	1012.85	56.97	<0.001	<0.001	0.746
CP-Total, g	342.97	723.11	1027.27	1296.34	66.06	<0.001	<0.001	0.777
CPNPN-Hay, g	93.26	97.69	87.40	76.78	14.42	0.622	0.117	0.988
CPNPN-Supplement, g	0.00	25.73	49.41	73.64	28.04	0.044	0.159	0.992
CPNPN-Total, g	93.26	123.42	136.81	150.42	22.88	0.037	0.286	0.995
TP-Hay, g	249.71	264.62	235.73	206.72	20.84	0.426	0.002	0.995
TP-Supplement, g	0.00	335.06	654.73	939.21	49.50	<0.001	<0.001	0.730
TP-Total, g	249.71	599.69	890.46	1145.93	57.58	<0.001	<0.001	0.755
TPHD-Hay, g	35.49	37.73	34.31	29.88	8.28	0.826	0.365	0.944
TPHD-Supplement, g	0.00	101.75	198.90	286.92	28.15	<0.001	<0.001	0.870
TPHD-Total, g	35.49	139.48	233.21	316.80	30.83	<0.001	<0.001	0.871

CP: crude protein; CPNPN: crude protein originating from non-protein nitrogen sources; TP: true protein; TPHD: highly available true protein. UNS: not supplemented; LOW: receiving 0.5% of body weight; MEDIUM: receiving 1.0% of body weight; HIGH: receiving 1.5% of body weight. Lower values p<0.05 denote statistical difference for the supplemented versus non-supplemented (S vs. UNS), linear (L), and quadratic (Q) contrasts. *Statistically different from control by Dunnett's test.

Consumption of low, slow, and zero digestibility nitrogenous fractions. When the consumption of low and slow availability and indigestible protein fragments was evaluated, no difference was observed between treatments in terms of the consumption of NDIP, ADIP, and TPSD from hay (p>0.05), and there was a linear downward effect on the consumption of NDIP and ADIP (p<0.05) as the level of supplementation increased (Table 3).

For the variables consumption of NDIP, ADIP, and TPSD of the supplement and total diet, there was a difference between supplemented treatments and UNS (p<0.05), with an ascending linear effect (p<0.05) observed between the supplemented treatments as the level of supplementation increased (Table 3).

Percentage composition of the consumption of crude protein. When the percentage of participation of the consumption of each protein fragment within the consumption of crude protein was measured, supplemented animals presented a higher concentration of TPHD/CP and TP/CP than non-supplemented (p<0.001), demonstrating an ascending linear effect as the amount of the supplement in the diet increased. Non-supplemented animals presented a higher CPNPN/CP concentration than supplemented

animals (p<0.001), with a linear decreasing effect observed between the supplement treatments as the supplementation level increased (p=0.014). No difference was found between supplemented and non-supplemented animals and between supplemented animals in the concentration of ADIP/CP, NDIP/CP, and TPSD (p>0.05) (Table 4).

Supplemented animals presented higher digestibility of the crude protein than non-supplemented (p<0.001), with an ascending linear effect (p=0.029) observed between the supplemented groups as the level of supplementation increased (Table 4).

Table 3. Consumption of slow and low availability and indigestible fragments, which are part of the crude protein present in hay and a supplement offered to Chino Santandereano cattle in stables.

Item	Treatments				SEM	p-value, Contrasts		
	UNS	Low	Medium	High		S vs. UNS	L	Q
NDIP-Hay, g	214.22	226.89	201.42	176.84	21.25	0.484	0.009	0.974
NDIP-Supplement, g	0.00	233.32	455.83	652.29	26.80	<0.001	<0.001	0.625
NDIP-Total, g	214.22	460.21	657.25	829.13	34.70	<0.001	<0.001	0.689
ADIP-Hay, g	177.81	188.27	168.18	147.39	15.83	0.460	0.005	0.972
ADIP-Supplement, g	0.00	168.04	327.00	476.14	39.80	<0.001	<0.001	0.904
ADIP-Total, g	177.81	356.31	495.18	623.53	39.68	<0.001	<0.001	0.892
TPSD-Hay, g	36.41	38.62	33.24	29.44	14.31	0.825	0.522	0.948
TPSD-Supplement, g	0.00	65.28	128.83	176.15	37.26	0.001	0.029	0.832
TPSD-Total, g	36.41	103.90	162.07	205.60	36.32	0.001	0.034	0.840

NDIP: protein insoluble in neutral detergent; ADIP: protein insoluble in acid detergent; TPSD: the difference between NDIP and ADIP. UNS: not supplemented; LOW: receiving 0.5% of body weight; MEDIUM: receiving 1.0% of body weight; HIGH: receiving 1.5% of body weight. p<0.05 denotes a statistically significant difference between the supplemented and non-supplemented (S vs. UNS), linear (L), and quadratic (Q) contrasts. * Statistically different from control by Dunnett's test.

Table 4. Percentage representation of the consumption of each protein fragment in relation to the consumption of crude protein and digestibility of crude protein.

Item	Treatments				SEM	p-value, Contrasts		
	UNS	Low	Medium	High		S vs. UNS	L	Q
TPHD/CP, %	10.55	19.22	22.55	24.25	2.20	<0.001	0.037	0.659
TP/CP, %	73.15	82.94	86.65	88.45	1.82	<0.001	0.014	0.563
CPNPN/CP, %	26.85	17.05	13.35	11.54	1.82	<0.001	0.014	0.563
ADIP/CP, %	52.08	49.29	48.36	47.96	2.25	0.078	0.602	0.904
NDIP/CP, %	62.61	63.73	64.10	64.21	2.97	0.573	0.867	0.956
TPSD/CP, %	10.53	14.43	15.74	16.24	4.16	0.171	0.668	0.912
Crude protein digestibility								
CPD, %	55.15	69.85	76.10	75.43	3.29	<0.001	0.029	0.098

%TPHD/CP: percentage representation of the consumption of highly available true protein, in the consumption of crude protein; %TP/CP: percentage representation of the consumption of true protein, in the consumption of crude protein; %CPNPN/CP: percentage representation of the consumption of crude protein originating from non-protein nitrogen sources, in the consumption of crude protein; %AIDP/CP: percentage representation of the consumption of protein insoluble in acid detergent, in the consumption of crude protein; %NDIP/CP: percentage representation of the consumption of insoluble protein in neutral detergent, in the consumption of crude protein; %CPD: percentage of digestibility of crude protein; UNS: not supplemented; LOW: receiving 0.5% of body weight; MEDIUM: receiving 1.0% of body weight; HIGH: receiving 1.5% of body weight. p<0.05 denote statistically significant difference for the supplemented versus unsupplemented (S vs. UNS), linear (L), and quadratic (Q) contrasts. * Statistically different from control by Dunnett's test.

DISCUSSION

The digestibility of crude protein in the diet of supplemented animals is normally higher than that of non-supplemented animals (11,12). This observation is commonly attributed to the supplementation providing greater amounts of ruminal ammoniacal nitrogen (RAN), in some cases up to 9.2 mg RAN/dL of ruminal fluid (amount provided by diets with a CP concentration of 124 g/kg DM) (13). being used by ruminal microorganisms to increase in number, generating, in turn, an increase in the degradation of proteins and other nutritional components that are part of the diet. This cycle increases in intensity.

in supplemented animals. Our control treatment was fed with hay with protein concentrations of 58.51 g/kg of DM, compromising the production of RAN and the digestibility of crude protein. Under these conditions, catabolic processes initiate the degradation of the nitrogen retained at the body level, promoting the mobilization of myofibrillar proteins, mainly in an attempt to contribute ammonia to the ruminal environment, which is associated with a reduction in animal production (1).

This evidence raises several questions on the relationship between RAN and digestibility, including on what does it depend on that food contributes a greater amount of RAN? The answer may not be related to the "quantity" effect since the concentrates provide the greatest contribution of CP and the concentrations of each of the protein fractions within the CP. According to Sniffen (3), the crude protein of food has several fractions that vary in digestibility, with CPNPN and TPHD being highly digestible, generating a greater amount of RAN, and TPSD and ADIP of slow degradability and indigestible, respectively, providing less RAN or even a RAN deficit. It is possible to consider, then, that, at a normal passage rate, the digestibility of the protein depends not only on the population of ruminal microorganisms but also on the concentration of said nitrogenous fractions in the crude protein, being able to find different CP digestibilities in animals with equality in the rumen population.

According to Sniffen (3), pastures and some agro-industry by-products have TPHD concentrations of no more than 5%/CP, while concentrates can double this value. Our results also report a higher concentration of TPHD in a supplement than in *Brachiaria humidicola* hay, yet, our hay and supplement concentrations were higher than those reported by Sniffen (3) with 10.546%/CP and 28.135%/CP, respectively.

In an investigation by Gaviria (14), grasses (*Megathyrsus maximus* [Jacq.] and *Cynodon plectostachyus* [K. Schum.]) presented concentrations of fraction A + B1, B3, and C of 34%, 27.7%, and 7.4% respectively. In the present study, higher concentrations of highly digestible fractions (A + B1) were observed with 37.392% and indigestible 52.082%, but a lower concentration of the fraction of slow degradability (10.527%).

Although ADIP represents the protein linked to the ADF (3,10), high concentrations of ADIP in hay can be explained by the increase in the concentration of ADF in the dry matter of this food. This increase generates at the same time a reduction in the concentration of the TPSD, as this is determined by the difference between NDIP and ADIP, with ADIP being 83% of the NDIP and the ADF being 98.62% of the NDF in the hay evaluated in this study. A study including low-quality grasses (*Brachiaria decumbens* Stapf) reported corrected NDF values for ash and protein (NDFap) of 80.1% in the DM, similar values to those observed in the present study, with 80.99% of NDFp in the MS. These data are expected, considering that the hay used was from deferred *Brachiaria humidicola*, harvested with a high regrowth age and in a reproductive state (1). Based on these results, it is possible to confirm that the concentrations of the nitrogenous fractions inside the crude protein vary and are dependent on the type of grass and age of the plant at the time of collection, as occurs with the concentration of macronutrients.

According to (1), using grass with 5% protein and 80.1% NDFap in the DM (perhaps with a large part of the protein attached to fiber), non-supplemented animals presented maximum RAN concentrations of 5.8 mg/dL four hours post-ingestion and digestibility of crude protein of 24.3%. These values are lower than those observed in animals that received 100% of the requirements of rumen degradable protein (RDP) and not rumen degradable (RDNP), showing 35.6 mg/dL RAN four hours post-ingestion and 81.1% protein digestibility.

Based on the above, we can affirm that the low digestibility of the CP observed in this study in non-supplemented animals can be explained by lower consumption of highly digestible

nitrogenous fractions that favor the contribution of RAN (4), perhaps affecting the population of fibrolytic and proteolytic bacteria, as fiber and protein are the dietary factors determining the community of host-microbial species (15) present. However, supplemented animals presented an increase in the consumption of highly digestible protein fractions as well as slow digestibility. An increase in the consumption of the TPSD fraction would represent the amount of protein that escapes ruminal degradation and could lead to an increase in the blood concentration of MP, greater retention of body nitrogen, and productive performance (1).

Increase in the consumption of CP, CPNPN, TP, TPHD, TPSD, NDIP and ADIP in the total diet of supplemented animals can be explained by the supplementation presenting high concentrations of these nutritional compounds, or perhaps reflecting a possible increase in the digestibility of the fibrous components present in the diet (8). Despite the higher concentration of CPNPN in hay than a supplement, the low digestibility of crude protein observed in non-supplemented animals due to high ADF concentrations in the hay may explain the lower consumption of CPNPN in non-supplemented animals than in supplemented animals.

In conclusion, the supplement improved the contribution of highly digestible and slow digestible protein fractions to the total diet supplied to Chino Santandereano cattle in stables. The supplementation increased the consumption of all the nitrogenous fractions present in the crude protein, and the digestibility of the crude protein of the total diet of the cattle by providing a greater quantity of highly digestible and/or usable nitrogen fragments that generate elevated ammoniacal nitrogen at the ruminal level.

Recommendations include utilizing this type of fractionation in routine laboratory analysis in animal nutrition research, mainly when using alternative or unconventional raw materials for which there is little information and determining the concentration of these protein fractions in food harvested at different regrowth ages.

Conflict of interests

We wish to confirm that there are no conflicts of interest associated with this publication (political, personal, professional) and that there has been no financial support that may have influenced the results. Furthermore, we declare that the manuscript does not contain material (images, tables, and others) reproduced from other publications and websites.

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