

INFLUENCE OF LIVE WEIGHT ON STARCH AND N DIGESTION OF CORN-BASED (DRY-ROLLED OR STEAM-FLAKED) FINISHING DIETS FOR HOLSTEIN STEERS †

[INFLUENCIA DE PESO VIVO SOBRE DIGESTIÓN DE ALMIDÓN Y NITRÓGENO DE LAS DIETAS DE FINALIZACION A BASE DE MAÍZ (ROLADO EN SECO U HOJUELEADO A VAPOR) PARA NOVILLOS HOLSTEIN]

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SUMMARY

Background. A possible effect of live weight on digestion capacity and starch and nitrogen utilization in cattle has been suggested, however, to our knowledge, this effect has not been evaluated by direct testing. This becomes especially relevant when finishing diets are used, which are characterized by their high percentages of starch and nitrogen **Objective:** To study the possible effect of live weight (BW) on digestion and utilization of two treatments corn, dry rolled (DR) and steam-flaked (SF). **Results**. Microbial N and microbial efficiency were not affected by the live weight by corn processing interaction. PV decreased linearly (P<0.01) rumen starch digestion in response to dry-rolled corn, whereas with steamed corn, PV had an opposite effect by linearly (P<0.01) increasing rumen starch digestion. **Implications.** Attention should not be paid to the live weight does not have a significant effect on the digestion of corn starch, but there is a quadratic effect on the nitrogen efficiency; however, the direction of this effect (increase or decrease in digestion as PV increases) depends on the type of grain processing. http://orcid.org/0000-0001-9509-406X

Key words: live weight; starch; digestion; corn processing.

RESUMEN

Antecedentes. Se ha sugerido un posible efecto del peso vivo sobre la capacidad de digestión y utilización de almidón y nitrógeno en el ganado bovino, sin embargo, hasta donde sabemos, este efecto no ha sido evaluado mediante pruebas directas. Esto cobra especial relevancia cuando son utilizadas dietas de finalización, las cuales se caracterizan por sus altos porcentajes de almidón. **Objetivo.** Estudiar el posible efecto de peso vivo (PV) sobre digestión y utilización en respuesta a dos tratamientos de maíz, rolado en seco u hojueleado a vapor. **Resultados.** El N microbiano y la eficiencia microbiana no se vieron afectados por la interacción del peso vivo y procesamiento del maíz. El PV disminuyó linealmente (P<0.01) la digestión ruminal del almidón en respuesta a maíz rolado en seco, mientras que con maíz hojueleado al vapor, el PV tuvo un efecto opuesto al aumentar linealmente (P<0.01) la digestión ruminal del almidón. **Implicaciones**. No se debe prestar atención al peso vivo de los animales al momento de elegir el tipo de procesamiento del grano. **Conclusiones.** Se concluye que el

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peso vivo no tiene un efecto significativo en la digestión del almidón del maíz, pero si un efecto cuadrático sobre la eficiencia del nitrógeno; sin embargo, la dirección de este efecto (aumento o disminución de la digestión a medida que aumenta la PV) depende del tipo de procesamiento.

Palabras clave: peso vivo; almidón; digestión; procesamiento de maíz.

INTRODUCTION

More than two thirds of the cost of feeding in feedlots can be attributed to grain costs (Surathkal and Madan, 2019). For this reason, it is important to take maximum nutritional potential of the grains through the most appropriate processing in order to obtain an optimal starch utilization and productive performance of livestock (Owens and Soderlund 2006). Dry rolled (DR) and steam-flaking (SF) are the most common processing for corn grain, steam flake corn increases total tract and ruminal starch digestion González-Vizcarra et al. (2017). Depending on the size of feedlot, SF could represent approximately 5 to 7-fold more cost than DR processing (Macken et al., 2006). Although, due to the increases in feed efficiencies observed in cattle that were fed with SF corn (by increases in starch and N digestion), SF processing is at present profitable (Owens et al., 1997; Zinn et al., 2002). However, there are suggestions of a possible effect of live weight (which is strongly associated with age in feedlot industry) on the capacity of digestion and utilization of starch in cattle. Zhang et al. (2023) suggest that chewing behaviour could influence total digestion by affecting saliva production via the masticatory-salivary reflex and subsequently, the fluid inflow to the rumen by contributing to particle size reduction. In this sense, it is expected that older cattle have a more efficient chewing mechanism (Reinhardt et al., 1998). However, Rainey et al. (2003) assert that young cattle tend to digest a greater percentage of raw grain starch than adult cattle. According to the above, Pordomingo et al. (2002) reported that the fraction of corn grain recovered from feces was 5.8% lower (P<0.01) in 155 kg steers compared to steers weighing 269 kg (diet contained 72% of whole grain) this suggests greater efficiency in lighter animals. In the same manner, Zinn et al. (1995) concluded that corn processing is a primary factor influencing site and extent of starch and N digestion. Therefore, some researchers mentioned that processing is justified for some types of grain and for a certain group of animals (Stritzer and Gingins, 1983). When decision is taken among grain processing methods, farmers must consider not just the nutritional value, but also the costs associated with processing, management and benefits (Zinn et al., 2002). Regarding the effect of live weight of cattle, and corn processing on the efficiency of starch utilization, to our knowledge,

this effect has not been assessed by direct testing. Thus, the objective of the present experiment was to evaluate the influence of live weight on starch and N digestion in corn-based (dry-rolled or steamflaked) finishing diets.

MATERIALS AND METHODS

The trial was conducted during 18 months at the Ruminant Metabolism Experimental Unit of the Instituto de Investigaciones en Ciencias Veterinarias of the Universidad Autónoma de Baja California, 10 km south of Mexicali City in northwestern México (32° 40' 7" N and 115° 28' 10" W). The area is about 10 m above sea level and has Sonoran desert conditions (BWh classification according to Köppen).

All animal management procedures were conducted within the following guidelines of locally approved techniques for animal use and care: NOM-051-ZOO-1995: Humanitarian care of animals during their mobilization; NOM-062-ZOO-1995: Technical specifications for the care and use of laboratory animals, livestock, farms, production, breeding and breeding centres, zoos and exhibition halls must comply with the basic principles of animal welfare; and NOM-024-ZOO-1995: Stipulations and characteristics of animal health during transport. These regulations are in accordance with the specific principles and guidelines presented in IACUC-290-30 and by the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies [FASS] 2010).

Six Holstein steers, habilitated with "T" type cannulas in the rumen and proximal duodenum (6 cm from pyloric sphincter) were used in a series of four experiments under a complete randomized design with repeated measures, to evaluate the influence of live weight (four weight categories) on the starch digestibility of finishing diet contained 71% DM basis. Having two treatments of grain, dry rolled corn (DR) TMT1 and steam-flaked corn (SF) TMT2, these were randomly assigned to the six experimental units. For the above, four weights were used 160 ± 10 kg, 250 ± 15 kg, 360 ± 14 kg and 440 ± 20 kg. Diet composition is shown in Table 1. Dry rolled corn was prepared by passing whole corn through rollers (46×61 cm rolls, 5.5

corrugations/cm; Memco, Mills Rolls, Mill Engineering & Machinery Co., Oklahoma, CA) that had been adjusted so that kernels were broken to a density of 0.50 kg/L. Steam-flaked corn was prepared as follows: A chest situated directly above the rollers (46×61 cm rolls, 5.5 corrugations/cm; Memco, Mills Rolls, Mill Engineering & Machinery Co., Oklahoma, CA) was filled to capacity (440 kg) with whole corn and brought to a constant temperature (102° C) at atmospheric pressure using steam (boiler pressure 60 psi). The corn was steamed for 20 min before starting the rollers. Approximately 440 kg of the initial steamprocessed grain that exited the rolls during warmup was not fed to steers on this study. Tension of the rollers was adjusted to provide the indicated flake density (0.31 or 0.36 kg/L). Retention time of grain in the steam chamber was approximately 18 min. The SFC was allowed to air-dry (5 d) before use in diet preparation. Yellow corn used was a commercial blend of US #2 dent. Cattle were weighed in an electronic single-animal weigh cage (Fairbanks scales, Kansas City MO, USA) at the start each experiment. During the lapsed period between experiments steers were fed with the SFcorn based diet at level of 2.2% of their BW. The experiments were started when steers reached the target weights of each experiment. The lapse of time between each experiment averaged 93±15 days. Steers were placed in individual pens (3.9 m2) which had a concrete floor covered by neoprene carpet, shared automatic drinkers water and individual feeders all of this in indoor facilities. Dry matter intake was restricted to 2.2% of LW. Diets were fed in two equal proportions at 0800 and 2000 daily. Chromic oxide (320 g air-dry basis/ton of diet), was premixed with minor ingredients (urea, limestone and trace mineral salt) before incorporation into complete mixed diets as an inert marker for calculating DM flow to the small intestine and fecal DM excretion.

Experimental periods consisted of a 10-d diet adjustment period followed by a 4-d collection period. During the collection period duodenal and fecal samples were taken from all steers, twice daily as follows: d 1, 0750 and 1350; d 2, 0900 and 1500; d 3, 1050 and 1650; and d 4, 1200 and 1800 h. Individual samples consisted of approximately 500 mL duodenal chyme and 200 g (wet basis) of fecal material. Samples from each steer and within each collection period were composited for analysis. Upon completion of the trial, ruminal fluid was obtained from all steers and composited for the isolation of ruminal bacteria via differential centrifugation (Bergen *et al.*, 1968). The microbial isolate served as the purine: N reference for the

estimation of microbial N contribution to chyme entering the small intestine (Zinn and Owens, 1986). Feed, duodenal, and fecal samples were subject to the following analysis: Dry matter (oven drying at 105°C until no further weight loss; method 930.15, AOAC 2000); ash (method 942.05, AOAC 2000); Kjeldahl N (method 984.13, AOAC 2000); chromic oxide (Hill and Anderson 1958), and starch (Zinn, 1990). Ammonia-N (method 941.04, AOAC 2000) and purines (Zinn and Owens 1986) were determined in duodenal samples.

Table 1. Ingredient and nutrient composition of experimental diets.

Ingredient	Dry	Steam-
composition	rolled	flaked
(% DM)	corn	corn
Dry-rolled corn	71.00	
Steam corn flake		71.00
Dry distillers grains	14.50	14.50
Cotton husk	12.00	12.00
Trace mineral salt ¹	2.50	2.50
Nutrient composition, I	DM basis ²	
Net energy (Mcal/kg)		
Maintenance	1.98	2.09
Gain	1.33	1.42
Crude protein (%)	11.63	11.23
Calcium (%)	0.3190	0.31
Phosphorous (%)	0.49	0.49

¹Essential Minerals content: CoSO4, 0.068%; CuSO4, 1.04%; FeSO4, 3.57%; ZnO, 0.75%; SO4, 1.07%; KI, 0.52%; and NaCl, 93.4%.

²Based on tabular values for individual feed ingredients (NASEM, 2000).

Organic matter of feed and digesta samples was estimated as the difference of DM minus ash content. Microbial organic matter (MOM) and microbial nitrogen (MN) entering to duodenum (measured from a duodenal cannula placed 6 cm from the pyloric sphincter) were calculated using purines as a microbial marker (Zinn and Owens 1986). Organic matter fermented in the rumen was considered equal to the OM intake minus the difference between the amount of total OM reaching the duodenum and the MOM reaching the duodenum. Feed N escape to the small intestine was considered equal to the total N leaving the abomasum minus ammonia-N and MN and, thus, includes any endogenous contributions. Ruminal microbial efficiency was estimated as duodenal MN, g/kg OM fermented in the rumen and protein efficiency represent the duodenal non-ammonia-N, g/g N intake.

Statistical analysis

Data were analyzed as a completely random design experiment with repeated measurements using the MIXED procedure SAS 9.3 statistical package. The covariance structure that was used for the records repeated in the time of k-th repetition in the model was selected from among the structures: and unstructured "UN", compound symmetry "CS" and autoregressive structure First-order with heterogeneous variance "AR (1)". The selection was made based on the one that produced values close to zero for the Akaike information criteria and Schwarz Bayesian criteria. The factors to be evaluated are the processing method effect and the body weight, because the interaction was not significant, it was removed from the model to increase the sensitivity of the processing method affects and body weight. An orthogonal polynomial orthogonal contrasts analysis was performed to measure the linear, quadratic and cubic effect of the interaction live weight× and grain processing and even Tukey's mean comparison test to compare animal weight and grain processing.

The statistical model of this experiment is as follows:

 $Yijk = \mu + \tau i + \alpha k (i) + Pj + \epsilon ijk$

Where: Yijk = response variable associated with a digestive parameter

 μ = where μ is the common experimental effect

 $\tau i = processing method effect.$

 αk (i) = effect associated with the repetition within the treatment.

Pj = effect of the body weight.

 ε ijk = is the residual error

In all cases, least squares means and standard error are reported and contrasts were considered significantly when the *P* value was ≤ 0.05 , and tendencies are identified when the *P* -value was > 0.05 and ≤ 0.10 .

RESULTS AND DISCUSSION

The effects of LW on site and extent of digestion of diets contained dry-rolled or steam flaked corn are shown in Table 2.

Although Rainey *et al.* (2003) assert that young cattle tend to digest a greater percentage of raw grain starch than adult cattle, there were no effects of LW and processing method on ruminal,

postruminal or total tract digestion of OM and N. In the same manner, microbial N and microbial efficiency were not affected by LW. However, with dry rolled corn, as LW increased, ruminal digestion of starch decreased (linear component, P<0.01), while with steam-flaked corn, LW had an opposite effect increasing linearly (P<0.01) ruminal digestion of starch (interaction P<0.01) as LW increased. This effect of LW was not observed on postruminal and total tract starch digestion. This result is uncertain, but may be related to chewing efficiency and particle size of corn grain. Related to the chewing efficiency according to the age of steers, in weaned calves, minor or nil advantages have been observed in response to corn processing, which may be due to conditions such as differences in relation to the extent of chewing (Loerch and Fluharty, 1998). On the same way, with the aim to determine the efficiency of mastication, Stritzer and Gingins (1983) evaluated the quantity of whole grain arrived to the rumen in light (325 kg LW) and heavy (596 kg LW) cattle. The percentage of whole grains (of total grains intake) that arrived to the rumen was 24.4 and 58.4%, for light and heavy cattle, respectively. They concluded that the efficiency of mastication of whole grains decreases as increase the animal live weight. Pordomingo et al. (2002) reported that the fraction of corn grain recovered from feces was 5.8% lower (P<0.01) in 155 kg steers compared to steers weighing 269 kg (diet contained 72% of whole grain) this suggests greater efficiency in lighter animals. However, the nature of the material seems to affect the efficiency of chewing in cattle. For example, to cell walls, Bae et al. (1983) indicate a positive relationship between live weight and chewing efficiency. For corn grain, the efficiency of chewing could be related to the particle surface area. Reinhart et al. (1998) reported that a greater surface area per gram of grain, increases chewing efficiency. Those researcher, indicate that the even when there were no differences on ruminal starch digestion (74% of corn in diet), heavier cattle (361 kg LW) shown a greater total tract digestion of starch from rolled corn grain than whole corn grain compared to lighter cattle (206 kg LW). Contrary to the above, Plascencia et al. (2003) observed in Holstein cattle that were fed a diet contained an average of 74% steam-flaked corn a significant lower (2.8%) ruminal starch digestion for heavy steers (370 kg LW) than for light steers (175 kg LW) without differences between both weight categories on total tract starch digestion. As mentioned above, in the present experiment with dry rolled corn, LW decreased (linear component, P<0.01) ruminal digestion of starch, while with steam-flaked corn,

Live weight (kg)									
	16	50	250 360		440		SEM		
Item	DRC	SFC	DRC	SFC	DRC	SFC	DRC	SFC	
Intake (g d ⁻¹)									
Dry matter	3.478	3.362	5.106	5.050	7.446	7.525	9.033	8.742	
Organic matter	3.232	3.142	4.796	4.777	6.974	6.927	8.489	8.222	
Starch	1.545	1.481	2.480	2.399	3.205	3.421	4.215	4.359	
Ν	71.0	65.0	101	95.9	154	148	177	160	
Flow to the duod	lenum (g	d ⁻¹)							
Dry matteraeh	1.721	1.553	2.691	2.380	3.994	3.640	4.436	3.813	220
Organic	1.228	1.035	1.907	1.499	2.902	2.381	3.160	2.358	159
matter ^{ad}									
Starchacd	319	231	503	221	815	437	1070	306	62.6
N^{ab}	60.2	57.9	94.2	100	141	151	148	161	1.3
Ammonia N ^{ab}	2.41	1.96	3.72	3.10	7.30	6.97	6.39	6.15	0.44
Non-ammonia	57.9	55.9	90.5	96.9	134	144	142	154	3.9
N^{ab}									
Microbial Nabe	23.56	24.17	39.49	46.79	54.99	62.10	64.75	78.45	1.2
Feed N ^{ab}	34.24	31.78	51.02	50.14	79.14	82.32	77.45	75.92	4.2
Ruminal digestic	on (%)								
Dry matter ^e	50.5	53.8	47.3	52.9	46.4	51.6	50.9	56.4	1.0
Organic matter ^d	64.7	69.2	62.6	70.3	61.0	68.4	65.0	73.0	1.1
Feed N ^b	51.7	51.2	49.4	47.8	48.6	44.4	56.1	52.5	1.0
Starch ^d	79.3	84.4	79.7	90.8	74.6	87.2	74.6	93.0	1.5
Microbial	11.75	11.48	13.68	14.34	13.66	13.59	12.17	13.49	0.4
efficiency ^{fh}									
Nitrogen	0.81	0.86	0.89	1.01	0.87	0.98	0.81	0.97	0.01
efficiency ^{beg}									
Fecal excretion,	g d ⁻¹								
Dry matter ^a	831	651	1313	998	1764	1382	1917	1745	114.7
Organic matter ^a	729	547	1161	864	1578	1154	1712	1456	100.7
Starch ^{adc}	126	11.9	267	10.6	330	14.1	417	26.8	33.9
N ^a	21.6	19.3	36.5	31.7	48.7	44.4	48.6	49.3	3.2
Postruminal dig	estion (%)							
Dry matter	51.4	57.9	51.5	57.9	55.1	61.1	55.5	55.1	2.1
Organic matter	50.0	57.0	49.8	56.0	53.2	61.1	53.7	54.7	2.1
Starch ^d	60.8	95.1	47.9	95.1	58.7	96.5	59.8	91.9	4.3
Ν	64.20	66.8	61.4	68.2	64.9	70.5	67.2	69.5	1.6
Total tract digestion (%)									
Dry matter	76.0	80.7	74.3	80.2	76.3	81.6	78.8	80.0	1.0
Organic matter	77.4	82.6	75.8	81.9	77.4	83.4	79.8	82.3	0.99
Starch ^d	91.9	99.2	89.2	99.6	89.7	99.6	90.1	99.4	1.0
Ν	69.6	70.4	63.8	67.0	68.4	70.0	72.5	69.2	1.5

Table 2. Influence of live weight and corn processing on characteristics of digestion.

^a Live weight linear effect, P < 0.01. ^b Live weight quadratic effect, P < 0.01.

^c Live weight by corn processing interaction, P < 0.01.

^d Corn processing effect, P < 0.01.

^e Corn processing effect, P < 0.05

^f Duodenal microbial N, g/kg OM fermented in the rumen.

^g Duodenal non-ammonia N, g/g of N intake.

^h Live weight quadratic effect, P < 0.05

LW had an opposite effect by increasing linearly (P<0.01) ruminal digestion of starch (P<0.01). Rainey *et al.* (2006) and Gorocica-Buenfil *et al.* (2005) did not detect effect of age of cattle and grain processing (P> 0.10) on starch digestion. They mention that the difference in the age of cattle (weaned vs. yearling) may not have been sufficient to allow the expression of the ability to chew. As expected, increasing LW increased DM intake and duodenal flows of compounds. But expressed as percentage of compounds flowing to duodenum,

similar to our results, Gorocica-Buenfil *et al.* (2005) did not observed LW effects on ruminal, postruminal or total tract digestion of OM and N. In opposite, Rainey *et al.* (2006) indicate a greater ruminal digestibility of N and OM for yearlings (385 kg LW) than for calves (310 kg LW).

The effects of corn processing on site and extent of digestion of diets contained dry-rolled or steam flaked corn are shown in Table 3.

Item	DRC	SFC	SEM	P-value
Intake (g d ⁻¹)				
Dry matter	6.266	6,170		
Organic matter	5.873	5.773		
Starch	2.861	2.915		
Ν	125.6	117.2		
Flow to the duodenum (g d ⁻¹)				
Dry matter	3.210	2.846	67.9	0.02
Organic matter	2.299	1.818	62.0	< 0.01
Starch	677	299	33.7	< 0.01
Ν	111	117	2.5	0.15
Ammonia N	4.96	4.54	0.15	0.13
Nonammonia N	106	113	2.5	0.14
Microbial N	45.7	52.9	1.7	0.04
Feed N	60.48	60.04	2.1	0.88
Ruminal digestion (%)				
Dry matter	48.8	53.7	0.83	0.014
Organic matter	63.3	70.2	0.92	< 0.01
Feed N	51.5	49.0	1.5	0.31
Starch	77.1	88.9	1.0	< 0.01
Microbial efficiency ^a	12.81	13.22	0.49	0.58
Nitrogen efficiency ^b	0.85	0.95	0.02	0.02
Fecal excretion, g d ⁻¹				
Dry matter	1457	1194	150	0.28
Organic matter	1295	1005	128.7	0.19
Starch	285	15.9	27.9	< 0.01
Ν	38.8	36.2	4.1	0.67
Postruminal digestion (%)				
Dry matter	53.4	58.0	4.3	0.49
Organic matter	51.7	57.2	4.3	0.42
Starch	56.8	94.7	3.3	< 0.01
Ν	64.4	68.7	3.3	0.41
Total tract digestion (%				
Dry matter	76.4	80.6	2.0	0.22
Organic matter	77.6	82.6	1.9	0.15
Starch	90.2	99.4	1.0	< 0.01
Ν	68.6	69.1	3.0	0.91

Table 3. Effect of corn pro	ocessing method	on digestion.
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^a Duodenal microbial N, g/kg OM fermented in the rumen.

^b Duodenal non-ammonia N, g/g of N intake.

Compared to DRC, steam flaking increased 13.6% (P=0.04) duodenal flow of MN and 11.1% (P=0.02) N efficiency (duodenal non-ammonia N, g/g of N intake). In previous studies no differences were detected by processing of corn on protein efficiency (Corona *et al.*, 2006), but in others (Zinn *et al.*, 1995; Barajas and Zinn, 1998) protein efficiency was greater for SFC than for DRC. In those cases, as happened here, the increase in protein efficiency was due to increased MN synthesis related to increased ruminal OM digestion.

Ruminal, postruminal and apparent total tract starch digestion for steam-flaked corn averaged 88.9, 94.6, and 99.4%, respectively. Those values are in good agreement with the average values summarized by Zinn et al. (2011). Consistent with previous studies (Owens and Zinn, 2005; Corona et al., 2006: Zinn and Owens, 2008: Plascencia et al., 2011) comparing steam-flaked vs. dry rolled corn, steam flaking increased (P < 0.01) ruminal (13.3%), postruminal (39.9%), and apparent total tract (9.25%) starch digestion. There were no effects (P>0.31) of corn processing on site and extent of N utilization. In a few reports (Corona et al., 2006, Plascencia et al., 2011), improvements due to steam flaking on postruminal and apparent total tract starch digestion had been associated with increased apparent postruminal and apparent totaltract N digestion. In as much as starch granules are encapsulated in a high UIP protein matrix comprising roughly 89% of corn protein (Allen and Ying, 2021), the association between increased post ruminal digestibility of starch and increased postruminal digestibility of N can be expected.

Compared with dry rolling, steam flaking increased ruminal (9.81%, $P = \langle 0.01 \rangle$ OM digestion. There were no effect (P > 0.10) of treatments on postruminal and total tract digestion of OM, notwithstanding that the postruminal and total tract digestion of OM were numerically greater by 9.6% and 6%, for SF compared to DRC, the difference were not statistically significant (P>0.10).

CONCLUSIONS

It is concluded that live weight has a significant effect on the starch digestion of corn; however, the direction of this effect (increasing or decreasing digestion as increasing LW) depends on the type of grain processing. With DR process, at greater weight, a lower starch digestibility, while SF processing showed the opposite effect. Processing the corn with steam increased the digestion of the starch by 9.3%. Live weight or corn processing did not affect the total tract digestion of nitrogen.

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Conflict of interest. The authors declare that there is no conflict of interest in this publication.

Compliance with ethical standards. This research was according to the regulations of the Committee of Ethics and Research of the Institute for Research in Veterinary Sciences-UABC and following the specific principles and guidelines presented in IACUC-290-30 and by the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies [FASS] 2010).

Data availability. The data are available with the corresponding author on request.

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