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IMPACT OF STARTER FEED AND FIBER COMBINATION ON RUMEN DEVELOPMENT AND PRODUCTIVITY IN NEONATAL LAMBS

Oziel Dante Montañez-Valdez[1], Alejandro Ley-de-Coss[1,2]*, Reynerio Bran[2a], Cándido Enrique Guerra-Medina[1,3], Ricardo Vicente-Pérez[4], and Miguel Chávez-Espinoza[1a]

[1] Grupo de Investigación en Nutrición Animal (GINA). Centro Universitario del Sur, Universidad de Guadalajara, Ciudad Guzmán. C. P. 49000, Jalisco, Mexico
https://orcid.org/0000-0001-9539-6623

[1a] Grupo de Investigación en Nutrición Animal (GINA), Universidad de Guadalajara, Centro Universitario del Sur, Ciudad Guzmán. C. P. 49000, Jalisco, Mexico
https://orcid.org/0000-0001-7293-3908

[2] Facultad de Ciencias Agronómicas, Campus V. Villaflores, Universidad Autónoma de Chiapas. C. P. 30470 Chiapas, Mexico
https://orcid.org/0000-0001-5982-300X

[2a] Facultad de Ciencias Agronómicas, Campus V. Villaflores, Cuerpo Académico Recursos Genéticos Tropicales, Universidad Autónoma de Chiapas. C. P. 30470, Chiapas, Mexico
https://orcid.org/0000-0002-5959-0709

[3] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro de Investigación del Pacifico Sur, Rosario Izapa. Tuxtla Chico, C. P. 30890, Chiapas, Mexico
https://orcid.org/0000-0001-9943-0032

[4] Centro Universitario de la Costa Sur, Universidad de Guadalajara. Autlán de Navarro, C. P. 48900 Jalisco, Mexico
https://orcid.org/0000-0002-4559-3116

* Corresponding author: alejandro.ley@unach.mx

ABSTRACT

The feeding of neonatal lambs in the tropics is based on milk and low-quality forages, which limits ruminal development and productivity. The objective of the study was to evaluate the rumen development and productivity of neonatal lambs raised under three dietary regimes: a) a liquid diet based on whole cow's milk (MILK); b) a solid diet based on fiber from low-quality forage, administrated by star grass (*Cynodon nlemfuensis*, FIBER); c) a solid diet based on the combination of fiber (40% FIBER and 60% starter feed, F40+SF60). Twelve 15-day-old lambs were evaluated over a 38-day period to assess: initial live weight, final live weight, dry matter intake, and daily weight gain, as well as the length, width, and number of ruminal papillae per cm^2; the volume of the reticulum, rumen, omasum, abomasum; ruminal pH; and concentration of total bacteria. A completely randomized design was used, with three treatments and four lambs per treatment. Data were analyzed using the PROC GLM procedure in SAS and means were compared using Tukey's test. In MILK, there was a greater number of rumen papillae in the cranial, ventral sac and ventral blind sac ($p < 0.05$); The number of papillae in the dorsal sac and dorsal blind sac, length and width of the ruminal papillae, reticulum volume and concentration of total bacteria were greater in F40+SF60 lambs ($p < 0.05$). Dry matter intake and daily weight gain were similar between the MILK and F40+SF60 dietary treatments ($p < 0.05$). The consumption of a combination of fiber and starter feed in neonatal lambs stimulated the development of rumen papillae, allowing weaning without affecting daily weight gain.

Keywords: Growth, nursing lambs, nutrition, microbiology, ruminants

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INTRODUCTION

Sheep feeding in tropical regions is primarily based on extensive grazing in grass-dominated pastures; however, the availability and quality of these pastures are affected by seasonal variations (Jarillo-Rodriguez et al., 2011). The nutrients most affected by seasonal changes include crude protein, neutral detergent fiber, and mineral content, alongside a reduction in the digestibility of the cell wall (Muñoz et al., 2016; Vieyra et al., 2013). In neonatal ruminants, the primary diet consists of maternal milk (Khan et al., 2016) and low-quality forage without any supplementation, which delays the functional development of the reticulum-rumen, reduces the ability to degrade forage nutrients, and often leads to diarrhea, adversely affecting weight gain and weaning weight (Gorka et al., 2009). Consequently, lambs raised without supplementation exhibit weaning weights of less than 12 kg at 60 days of age (Chay-Canul et al., 2019). Therefore, the feeding of neonatal lambs should be complemented with solid feed to improve productive performance (Hinojosa-Cuéllar et al., 2012; 2018). However, there is limited knowledge about the impact of solid feeding on ruminal development in early-weaned sheep. It is noteworthy that the type of feed and the nutrient content consumed by neonatal ruminants play a crucial role in the establishment and activity of ruminal microorganisms (Morgavi et al., 2010), ruminal development and functionality (Guerra-Medina et al., 2021), and consequently, the ability to utilize forage nutrients, which influences daily weight gain and weaning weight. Feeding strategies with starter diets during the first week of life in lambs allows a better physiological adaptation to solid feed, improved establishment of ruminal microbiota, and increased enzymatic activity (Bertan, 2016). The ruminal metabolism of rapidly fermentable carbohydrates promotes greater synthesis of fatty acids, including propionate and butyrate, which stimulate the development of ruminal epithelium in neonatal animals (Gorka et al., 2009; Klevenhusen et al., 2013; Abubakr et al., 2014). This increased ruminal activity reflects better productive performance and weaning weight, aiding in the reduction of weaning age (Rasby, 2007). In contrast, the intake of neutral detergent fiber (forage) promotes the development of the ruminal muscular wall, stimulates rumination, and enhances salivary flow to the rumen (Chaves et al., 2014). The combination of fiber and starter feed in the diet of neonatal lambs in the humid tropics enhances rumen development, promotes microbial population balance, and optimizes productive performance compared to diets based solely on starter feed. Therefore, this study aimed to evaluate the effect of the type of feed on ruminal development, microbial population, and productive response in neonatal lambs in the humid tropics.

MATERIALS AND METHODS

Location, general management, and experimental design

The experiment was conducted at the Centro Universitario de Transferencia de Tecnología (CUTT), and on the Agricultural Health Laboratory at the 'San Ramón' facility of the Faculty of Agricultural Sciences, Campus V, Autonomous University of Chiapas, located in Villaflores, Chiapas, México, at coordinates 16°27'59" LN and 93°28'43" WL. The prevailing climate is classified as warm sub-humid (AW1) (W)(i´) g, according to the Koppen and Thornthwaite climate classification (Yadav, 2024). The region has an average annual rainfall of 1200 mm, predominantly occurring from June to November, with an average temperature of 22 °C, and an altitude of 591 meters above sea level (CONAGUA, 2021).

Twelve 15-day-old neonatal Katahdin lambs, with an average body weight (BW) (± standard deviation) of 6.38 ± 0.345 kg, were subjected to early weaning and housed individually in pens (1.2 m^2), each equipped with feeders and automatic drinkers. The lambs were externally dewormed with 2% Fipronil (1 mg kg^{-1} BW percutaneously) and orally with albendazole (5 mg kg^{-1} BW), as parasites were presented in their mothers. During the first eight days of adaptation, the lambs were allowed to nurse from their mothers for 20 min in the morning as part of a progressive weaning process. During this period, four lambs were randomly selected as the control group and raised on a liquid diet based on whole cow's milk (MILK). The remaining lambs (n=8) had their liquid diet replaced with a solid diet. Four of these lambs were fed with star grass (FIBER) (*Cynodon nlemfuensis*, Vanderyst), while the other four received a diet containing 40% FIBER plus 60% of a starter feed (F40+SF60) formulated or lambs, providing 1.4 Mcal of Net Energy gain (NEg) and 18.5% crude protein (CP), as recommended by the NRC (2007) for sheep.

The lambs were fed their respective diets for 30 days, with feed provided ad libitum at 07:30 AM and 04:00 PM; water was freely available. The experimental conditions of the animals adhered to the Mexican Official Standard for technical specifications for the production, care, and use of laboratory animals (NOM-062-ZOO, 1999). A 1.0 kg sample of the solid diets was processed

to determine NEg based on the calculation from Solver Excel® (formulation software), while contents of dry matter (DM), crude protein (CP), ether extract (EE), calcium, phosphorus (AOAC, 2012), neutral detergent fiber (NDF), and acid detergent fiber (ADF) (Van Soest, 1991) were also determined. Additionally, a milk sample was assess to determine contents of CP, EE, Ca, P, and DM (Table 1).

Dry matter intake and daily weight gain

The lambs' daily intake of milk (mL day^{-1}) and solid diets (g day^{-1}) were recorded individually over a 30-day period. For the lambs on the MILK treatment, 810 and 788 mL of milk were provided at 7:30 and 4:00 PM, respectively, following the adaptation period. The milk was administered in bottles, allowing with ad libitum access for each animal. Lambs consuming the FIBER and 40F+60SF diets had free access to these feeds starting from the adaptation period. Daily dry matter intake was calculated as the difference between the offered feed and the feed refusal. Average daily weight gain was determined by weighing the lambs (pre-prandially, 08:00 AM) every seven days until the end of the sampling period.

Animal sacrifice and sample collection for analysis

On the final evaluation day (~ 53 days of age), all lambs from each treatment group were sacrificed 3 h postprandially. Prior to entering the slaughter line, each lamb was rendered unconscious by electrical stunning, in accordance with the Terrestrial Animal Health Code from the World Organization for Animal Health (WOAH, 2022). Subsequently, the lambs were exsanguinated by severing the carotid and jugular veins, following the Animal Welfare guidelines outlined in Article 7.5.1. of the WOAH. To assess the impact of the diet on rumen development, samples were taken via abdominal incision, and the digestive tract of the lambs was exposed. Each compartment of the stomach (reticulum, rumen, omasum, and abomasum) was weighed with and without its contents (feed) using an analytical balance (Electronic Scale, B2000t2, México). Morphometric measurements were taken for each compartment to determine its volume. Following the removal of gastrointestinal contents, ruminal wall samples were collected for histological evaluation using the microscopy technique described by Guerra-Medina et al. (2021).

Dissection and sampling for analysis

After exposing the compartments, the rumen was dissected using the technique described by Lesmeister et al. (2004). To evaluate rumen development, a symmetrical incision was made on both sides of the rumen, ensuring that the structural integrity of the organ was preserved. A modified version of this technique was then used for papillae sampling, with five specific sections of the rumen defined as areas of interest: the cranial sac (*saccus cranialis*), dorsal sac (*saccus dorsalis*), ventral sac (*saccus ventralis*), caudodorsal blind sac (*saccus caecus caudodorsalis*) and caudoventral

Table 1. Ingredients and chemical composition of diets for neonatal lambs.

Ingredients	Percentage of the diet		
	MILK	FIBER	F40+SF60
Whole Cow's Milk (MILK)	100	--	--
Star grass (FIBER)	--	100	40
Starter feed (SF)	--	--	60
Chemical composition, %			
Crude protein	3.57	7.6	12.62
Calcium	1.25	0.27	0.66
Phosphorus	0.28	0.22	0.37
Neutral detergent fiber	ND	67.24	45.70
Acid detergent fiber	ND	24.56	23.57
Ether extract	3.82	1.96	2.98
Dry matter	12.1	88.4	89.2
Net energy gain (Mcal kg^{-1})	ND	0.85	1.25

ND = Not determined. Starter feed (Corn, 59%; Soybean meal 44, 15%; Aminosoy (Bypass Protein), 8%; Wheat bran, 10%; Cane molasses, 2%; Calcium carbonate, 2%; Salmipro®, 2%; NaCl, 2%).

blind sac (*saccus caecus caudoventralis*) (Konig and Liebich, 2004). From each section, one tissue sample of approximately 1 cm² was collected from the central part, along with four additional samples from the corners. The samples were fixed in a 10% formalin solution for 24 h to preserve tissue structure and allow for further processing. Subsequently, the samples were washed and dehydrated using a graded series of alcohol up to 100% concentration. They were then cleared with xylene and toluene, followed by infiltration with molten paraffin using a tissue processor (Carl Zeiss; Model Myr, USA) and a semi-automatic microtome (Ecoshel; Model 355, Mexico) with three *µm*-thick sections, and a low-profile disposable blade to obtain 5.0 µm-thick sections. The sections were placed in inclusion cassettes (Simport®), embedded in blocks, and dried before histological processing, including embedding, microtomy, staining, and mounting of the tissue. Morphometric analysis was performed using a digital camera (Nikon, Kit D7500, Mexico) attached to a microscope, capturing five photographs of histological sections from each sample taken in the different areas of the rumen described above. These photographs were used to measure the length and width of the ruminal papillae for each lamb. The averages for all samples were calculated for each animal in each treatment. The count of ruminal papillae per treatment in each rumen section was conducted on a 1 cm² sample using a LABOMED Mod. LX400 optical microscope, USA, with a 10x/20 ocular and 4X objective lens.

Rumen bacterial concentration and ruminal pH

After the animals were sacrificed, ruminal fluid was obtained from the ventral part of the rumen using a 100 mL sterile syringe. The fluid was filtered through eight layers of sterile gauze to remove particles, collected in 50 mL vials, and the pH was measured with a pH meter (Orion A250, Orion Research, Inc., USA). To determine the ruminal bacterial concentration, 0.5 mL of ruminal fluid was inoculated in triplicate into a culture medium containing glucose-cellobiose-starch plus ruminal fluid (GCS+RF) to quantify bacterial populations. The medium was sterilized for 15 min at 121 °C and 15 psi (Autoclave All American 1925X, USA) and 4.5 mL of medium deposited in culture tubes (13×100 mm, PYREX®, México). The evaluation was conducted under CO_2 flow (99% purity, PRAXAIR®, México) sterile conditions in culture tubes using the most probable number (MPN) technique (Harrigan and McCance, 1990). The inoculated media were incubated at 38 °C for 24 h (Felisa FE-373, Fabricantes Feligneo, Mexico), with tubes exhibiting turbidity considered positive. The culture medium (GCS+RF) contained: 0.06 g of D-(+)-glucose + 0.06 g D-cellobiose + 0.06 g of starch (J. T. Baker); 30 mL of clarified ruminal fluid, which was centrifuged for 15 min at 17,664 g and sterilized for 20 min at 121°C and 15 psi.; 5.0 mL of mineral solution I [6 g K_2HPO_4 (Meyer) in 1000 mL H_2O]; 5.0 mL of mineral solution II [6 g KH_2PO_4 (Meyer) + 6 g $(NH_4)_2SO_4$ (Meyer) + 12 g NaCl (Meyer) + 2.45 g $MgSO_4$ (Meyer) + 1.6 g $CaCl_2 \cdot H_2O$ (Fermont) in 1000 mL H_2O], 2.0 mL of 8% Na_2CO_3 (Meyer) solution; 2.0 mL of sulfide-cysteine reducing solution [2.5 g L-cysteine (Sigma) in 15 mL of 2N NaOH (Meyer) + 2.5 g Na_2S-$9H_2O$ (Meyer) diluted to 100 mL with H_2O]; 0.2 g of trypticase peptone (MCD Lab); and 0.1 mL of 0.1% resazurin (Sigma-Aldrich) indicator solution.

Experimental design and statistical analysis

A completely randomized design was used, with three treatments and four lambs per treatment, with the following statistical model:

$$Yij = \mu + Ti + \varepsilon ij$$

Where: Yij = Response observed in the experimental unit j of treatment i, μ = General mean, Ti = Effect of the treatment i (diet), and εij = Error experimental.

Data for daily weight gain, ruminal pH, and the number, width, and length of papillae were analyzed using the PROC GLM procedure in the SAS statistical software package (2011). For the variable total bacterial concentration, the same model (PROC GLM) was used with the Kruskal-Wallis test, based on independent rank data (Wilcoxon). Means were compared using Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The number, length, and width of ruminal papillae in neonatal lambs are shown in Table 2. In lambs on the whole MILK treatment, there was a higher number of ruminal papillae in the cranial sac (p = 0.0025), ventral sac (p = 0.0148), and caudoventral blind (p = 0.0014) compared to the other treatments. However, in the caudoventral blind sac, the number of papillae was similar between the MILK and FIBER treatments. Conversely, the number of papillae in the dorsal sac (p = 0.0154) and caudodorsal blin sac (p = 0.0058) was higher in lambs on the 40F+60SF treatment. The length and width of the papillae were greater in all sections of the rumen in lambs on the 40F+60SF treatment compared to those on

Table 2. Variables of rumen development by anatomical section and type of diet consumed in 53-day-old neonatal lambs.

	Treatments				
	MILK	FIBER	40F+60SF		
Rumen Section	Number of rumen papillae (cm²)			SEM	Pr > F
Cranial sac	87.00a	45.00b	36.00b	16.42	0.002
Dorsal sac	0.00c	29.00b	41.00a	10.96	0.014
Ventral sac	92.00a	31.00c	50.00b	17.81	0.015
Caudodorsal blind sac	0.00c	28.00b	42.00a	11.05	0.005
Caudoventral blind sac	78.00a	40.00b	39.00b	14.54	0.001
Length of papillae (μm)					
Cranial sac	85.50c	291.90b	668.10a	115.86	0.014
Dorsal sac	0.00c	330.40b	714.40a	134.15	0.006
Ventral sac	97.70c	316.20b	609.70a	103.09	0.005
Caudodorsal blind sac	0.00c	311.60b	656.10a	123.82	0.016
Caudoventral blind sac	105.30c	304.70b	565.90a	94.17	0.011
Width of papillae (μm)					
Cranial sac	75.50c	256.90b	379.00a	65.63	0.006
Dorsal sac	0.00c	295.40b	425.30a	85.25	0.012
Ventral sac	87.70c	241.20b	335.60a	56.11	0.016
Caudodorsal blind sac	0.00c	276.60b	367.00a	75.67	0.003
Caudoventral blind sac	95.30c	269.70b	289.80a	50.06	0.002

[a, b, c]: Values with different letters in the same row are different (p <0.05). SEM: standard error of the mean. ND = Not determined.

MILK and FIBER diets ($p \leq 0.0158$). These results indicate that feeding neonatal lambs with starter feed leads to greater development of ruminal papillae compared to lambs being fed with a forage-based diet or a milk diet.

The stimulation of papilla growth is attributed to the fermentation of the starter feed, which increases volatile fatty acids (VFA) production, particularly butyrate, an essential energy source for ruminal development (Khan et al., 2016; ZeidAli-Nejad et al., 2017; Guerra-Medina et al., 2021; Pokhrel and Jiang, 2024). The greater length of papillae in lambs can be explained by increased chemical stimulation due to higher VFA production resulting from starter diet fermentation (Khan et al., 2008). Additionally, the transition from liquid to solid feed in weaned calves promotes papilla adaptation, improving nutrient absorption, developing a strong keratinized layer and forming a more abundant connective fibrous axis, which are typical features of functional rumens (Khan et al., 2011; Castro and Elizondo, 2012; Frandson et al., 2017).

Increased butyrate production during the fermentation of grain-based diets causes substantial changes in papillae growth and ruminal muscle development, with the physical stimulation generated by the solid feed contributing to increased muscle weight and papilla development (Kristensen et al., 2007; Bertan, 2016). These findings align with previous reports indicating that grain-based diets stimulate the growth of ruminal mucosa, with pre-ruminants fed with starter feed developing longer and wider papillae, as well as greater reticulum-rumen muscle weight (Kato et al., 2011; Suarez et al., 2011). This could explain the greater growth of ruminal papillae in lambs that consumed the 40F+60SF diet.

In measurements of digestive tract compartments (Table 3), the volume of the reticulum was similar in lambs from the FIBER and 40F+60SF treatments ($p > 0.05$), indicating that both diets promoted similar development in this compartment. The volume of the rumen and omasum was greater in animals that received the FIBER diet ($p < 0.0082$), which can be attributed to the higher content of fiber in this diet. Fiber-rich diets promote greater retention of material in the rumen, leading to distension and increased rumen wall stimulation, resulting in enhanced muscular development and compartment size (Dehority, 2003; Xie et al., 2013). The abomasum was larger in lambs on the MILK treatment ($p = 0.0026$), likely due to the higher volume of liquid ingested and processed in this compartment,

which is the primary digestive organ for milk in neonatal ruminants.

Regarding the concentration of total bacteria (Table 3), it was higher in the 40F+60SF treatment compared to the other treatments (p = 0.0064). This increase is attributed to the greater availability of fermentable nutrients and growth factors in the starter feed diet, which enhances bacterial proliferation, fermentative activity, and ruminal development. The pH of the ruminal contents (Table 3) was higher in lambs on the FIBER treatment, which is consistent with fiber fermentation generating less lactic acid and maintaining a more stable ruminal environment. However, despite the presence of starter feed in the 40F+60SF treatment, the ruminal pH remained at a favorable level (6.6), supporting optimal conditions for bacterial growth and fermentation (Vargas et al., 2023). The establishment, increase in population, and fermentative activity of ruminal bacteria in pre-ruminants require easily fermentable substrates, including soluble carbohydrates, NH_3, and growth factors such as B vitamins and organic acids. Studies have shown that microbial ruminal activity can be established as early as 10 days of age in pre-ruminants if the necessary conditions and substrates are present (Suarez et al., 2006; Belanche et al., 2007; Bradley, 2019).

Regarding the productive variables of the lambs (Table 4), there was no difference in initial live weight among treatments (p > 0.05). Final live weight was similar between lambs consuming the MILK and 40F+60SF diets and was higher compared to those on the FIBER diet (p = 0.0475). Likewise, daily weight gain was higher in lambs fed on MILK and 40F+60SF compared to those consuming the FIBER diet (p = 0.0215). These results suggest that early weaning can be performed without negatively affecting productive performance, provided that a high-quality starter feed is offered, as the response is suboptimal when only fiber is provided (Lui et al., 2022). Dry matter intake was similar between lambs consuming the 40F+60SF and FIBER diets (p > 0.05) and higher than in those receiving MILK (p = 0.0148).

Table 3. Volume of rumen compartments, rumen bacterial concentration, and ruminal pH in 53-day-old neonatal lambs.

	Treatments				
	MILK	**FIBER**	**40F+60SF**		
Compartment	**Volume of compartments (mL)**			**SEM**	**Pr > F**
Reticulum	150c	683a	704a	126.29	0.027
Rumen	350c	3740a	3024b	640.54	0.002
Omasum	175c	720a	520b	112.85	0.008
Abomasum	1758a	675c	840b	194.50	0.003
Per mL of rumen liquid content					
Total bacteria	1.6 x103c	1.3 x107b	1.5 x108a	2.7 x107	0.006
Ruminal pH	ND	6.8a	6.6b	1.29	0.003

[a, b, c]: Values with different letters in the same row are different (p <0.05). SEM: standard error of the mean. ND = Not determined.

Table 4. Summary of performance and body measurement variables for neonatal lambs.

	MILK	**FIBER**	**40F+60SF**	**SEM**	**Pr > F**
Initial Weight (kg)	6.85a	6.95a	6.95a	2.65	0.0687
Final Weight (kg)	11.84a	9.85b	12.00a	3.75	0.0475
Daily Weight Gain (g d^{-1})	178.00a	68.00b	180.00a	33.00	0.0215
Dry Matter Intake (g d^{-1})	192.51b	222.72ab	235.36a	11.35	0.0148

[a, b]: Values with different letters in the same row are different (p <0.05). SEM: standard error of the mean.

CONCLUSIONS

The consumption of starter feed by neonatal lambs promoted ruminal development and increased the population of total bacteria, due to the higher availability of nutrients and growth factors. This resulted in greater papillae length and width, enhancing ruminal functionality. Consequently, lamb fed starter feed achieved daily weight gain like those fed 100% milk and greater than those consuming 100% grass. These results suggest that early weaning can be performed without negatively affecting lamb productivity in tropical conditions, provided a high-quality starter feed is offered.

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Author contributions

All authors of the article actively contributed to the bibliographic review, the development of the methodology, the discussion of the results, and review and approval of the final version of the article.

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