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The Effects of Varied Neutral Detergent Fiber Levels on Blood Parameters, Nutrient Digestibility, Rumen Fermentation Characteristics and Rumen Tissue Morphology in Lambs

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ABSTRACT

Background: Neutral detergent fiber is a common analytical measure of feed fiber content, which could play an important role as an energy source, improve the rumen ecosystem and thus improve growth performance of lambs. The aim of this study was to investigate the effects of different levels of neutral detergent fibers on growth performance, blood parameters, nutrient digestibility, volatile fatty acid concentration, ammonia nitrogen, bacterial quantification, gas production and tissue morphology of growing lambs. **Methods:** Seventy-two Awassi lambs of weaning age were randomly assigned to three experimental feeds with different levels of neutral detergent fiber: low (210 g/kg), medium (280 g/kg) and high (350 g/kg), each with eight replicates in a completely randomized design. The growth performance parameters were evaluated over a trial period of 84 days. At the end of the trial, hematological and serum biochemical parameters and nutrient digestibility were evaluated. In addition, rumen fermentation characteristics, including pH, ammonia-nitrogen, gas production, volatile fatty acids, bacterial quantification and indices of rumen morphology indices were measured. **Result:** The results showed that lambs fed a medium-neutral detergent fiber feed had better growth performance ($p<0.05$). In addition, this experimental feed showed improvements in some blood parameters (WBC, RBC, HGB, TP, GLOB and GLU) and nutrient digestibility with the exception of crude protein and ash ($p<0.05$). Rumen fermentation characteristics and rumen morphology were also positively influenced by the medium- neutral detergent fiber level ($p<0.05$). These results indicate that a medium level of neutral detergent fiber (280 g/kg) is essential for optimal growth, health and rumen development in growing lambs.

Key words: Digestibility, Fermentation, Lambs, Morphology, Neutral detergent fiber, Performance.

INTRODUCTION

Dietary fiber is an important dietary component for ruminants, including growing lambs, as it plays an important role as an energy source and also improves the rumen ecosystem, nutrient digestibility and growth performance (Cannas *et al*., 2019). Neutral detergent fiber is a common analytical measure of feed fiber level content. They contain containing cellulose, hemicellulose and some lignin, which in turn can influence the amount of feed intake, the passage rate and thus the apparent digestibility of nutrients. (Carrillo-Díaz *et al*., 2022). Therefore, neutral detergent fibers in feeds play an important role in many ruminant nutrition studies (Flores-Mar *et al*., 2017). According to Alhidary *et al*. (2017) and Abdelrahman *et al*. (2018), feeding lambs with 300 g of neutral detergent fiber per kg of total mixed feed has positive effects on rumen fermentation characteristicstraits and improved growth performance parameters. Although fiber in general and neutral detergent fiber in particular are essential for rumen growth and function, high fiber content can reduce growth performance and feed efficiency, resulting in lower feed digestibility and increased satiety (Neave *et al*., 2018). Conversely, feeds with lower levels of neutral detergent fiber (below 200 g/kg) lead to rumen acidosis and other metabolic disorders that can affect the health and welfare of lambs (Pereira *et al*., 2016). Therefore, improving dietary fiber content is crucial for maximizing growth performance by improving lamb health, digestibility and metabolic and morphological characteristics of the rumen (Gallo *et al*., 2014; Asadollahi *et al*., 2017). However, one study reported that feeding lambs with 220 g neutral detergent fiber/kg of feed increased weight gain and improved growth performance without affecting feed intake (Mirzaei-Alamouti *et al*., 2021a).

The rumen is a complex ecosystem with a variety of bacteria that ferment fibers into volatile fatty acids, which are the primary source of energy for ruminants (Plaizier *et al*., 2022). Different levels of neutral detergent fibers and their sources influence rumen fermentation characteristics and

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growth performance of lambs (Wang *et al*., 2020). One study reported that lambs fed high levels of neutral detergent fiber stimulated cellulose-degrading bacteria, resulting in increased acetate concentration, while low levels stimulated amylolytic bacteria and increased propionate concentration (Holtzapple *et al*., 2022). Changes in fermentation and volatile fatty acid production play an important role in performance, rumen morphology and methane production in ruminants fed neutral detergent fibers (Ramos *et al*., 2021). In addition, lambs fed 250 g of neutral detergent fiber / kg of feed can improve fiber the digestibility of fiber due to increased microbial activity (Gallo *et al*., 2019). However, feeding optimal levels of neutral detergent fiber can stimulate rumen growth and development, resulting in improved rumen fermentation and morphology in growing lambs (Chen *et al*., 2024). Gallo *et al*. (2019) reported that feeding neutral detergent fiber to growing lambs had no effect on hema-biochemical parameters and short-chain fatty acid production. In contrast, Zhao *et al*. (2015) reported that lower levels of neutral detergent fiber increased glucose concentration in dairy cows.

Many studies have been conducted on the effects of dietary fiber on various aspects of ruminant physiology. The specific effect of optimal levels of neutral detergent fiber on lambs, including growth performance, digestibility, blood parameters, rumen fermentation and tissue morphology, remains a limited knowledge gap, as hypothesized in the present study. Therefore, this study aims to determine the optimal level of neutral detergent fiber in the feed of growing lambs by evaluating growth performance, blood parameters, nutrient digestibility, volatile fatty acid concentration, ammonia nitrogen, bacterial quantification, gas production and tissue morphology.

MATERIALS AND METHODS

Animals, design and feeds of experimental

The present study was conducted from 2024-01 to 2024-06 at the research station of the Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, Kingdom of Saudi Arabia. Seventy-two growing Awassi lambs of about three months of age were used for the present study. Lambs were randomly assigned to three experimental feeds in the form of pelleted complete feed with different levels of neutral detergent fiber: low (210 g neutral detergent fiber/kg feed), medium (280 g neutral detergent fiber/kg feed) and high (350 g neutral detergent fiber/kg feed), with eight replicates (pen, 3.5 m^2) per experimental feed (three lambs per replicate) using a completed randomized design. While neutral detergent fiber levels differed between the experimental feeds, all formulations met the other nutritional requirements such as protein, energy, vitamins and minerals recommended for growing lambs. The specific feed ingredients and nutrient composition of each experimental feed are shown in Table 1. The study was conducted for a 14-day acclimatization period followed by an 84-day experimental period. Feed and fresh water were constantly available and strict hygiene and ventilation standards were maintained throughout experimental period. All lambs were vaccinated against common diseases prevalent in Saudi Arabia, including parasites, peste des petits ruminants, septicemia, plague and enterotoxemia, following the guidelines of the Ministry of Environment, Water and Agriculture, Department of Animal Health.

Table 1: The specific feed ingredients and nutrient composition (% dry matter) of each experimental feed.

Ingredients, g/kg	Experimental feeds		
	Low	Medium	High
Yellow corn	351.4	264.5	289.8
Dates molasses	30.0	30.0	30.0
Wheat bran	180.0	208.0	200.0
Alfalfa hay	221.3	210.8	135.3
Wheat straw	70.0	70.0	40.1
Soy meal hulls	0.00	78.7	182.1
Soybean meal	98.0	102.5	92.3
Calcium carbonate	30.0	19.5	14.0
Sodium chloride	8.3	5.0	5.4
Sodium bicarbonate	8.0	8.0	8.0
Premix feed for lambs*	*3.0	3.0	3.0
Total	1000	1000	1000
Nutrient composition analysis, g/kg			
Dry matter	961.8	945.2	958.6
Organic matter	866.3	862.0	878.4
Crude protein	148.5	146.7	146.6
Neutral detergent fiber	210.0	280.0	350.0
Acid detergent fiber	138.2	130.8	170.1
Lignin	44.3	45.4	52.5
Starch	262.8	227.7	244.6
Fat	38.1	29.7	38.6
Ash	95.5	83.3	80.1
Calcium	29.5	19.6	17.4
Total phosphorus	3.1	4.2	4.1
Magnesium	4.2	3.2	3.9
Potassium	13.4	15.6	13.4
Sodium	6.7	2.8	3.2
Dichloride	15.6	5.6	6.8
Sulfur	2.8	2.3	2.3
Iron	0.50	0.72	0.43
Copper	0.01	0.01	0.01
Zinc	0.17	0.15	0.15
Metabolizable energy, kcal/kg	2850	2920	2940

*The vitamins and minerals per kg premix: vitamin A= 12,000 IU, vitamin D= 1,200 IU, vitamin E= 25 IU, magnesium= 400 mg, zinc= 70 mg, copper = 30 mg, manganese= 80 mg, selenium= 0.4 mg and cobalt = 0.8 mg.

Evaluation of growth performance indicators

During the entire experimental period of 84 days, lambs and feed per replicate were weighed every four weeks (1, 28, 56

and 84 days) after a 10-hour fast to monitor body weight (BW) and feed intake (FI). The data obtained were used to calculate total weight gain (TWG), daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) per replicate (pen) as an experimental unit, according to the experimental design of the present study. The calculation methods were as follows, according to the previously described method (Zhang *et al*., 2021; Rangamma Costa *et al*., 2022a; Tayeb *et al.,* 2023).

$$\text{TWG, kg} = \text{Final BW} - \text{Initial BW}$$

$$\text{FCR, g/g} = \frac{\text{DFI}}{\text{DWG}}$$

$$\text{DWG, g/day} = \frac{\text{TWG/1000}}{\text{Days of the study period}}$$

$$\text{DFI, g/day} = \frac{\text{Feed offered - Feed refused}}{\text{Days of the study period}}$$

Determine hema-biochemical parameters

Two blood samples (10 mL) were collected in collection tubes with or without K2 EDTA anticoagulant (Becton, NJ, USA) from one lamb per replicate (eight lambs per experimental feed) 1 hour before feeding by jugular venipuncture at the end of the experiment (84 days). All samples were stored at 4°C to determine the hematological and biochemical parameters according to the previously described method (Dorantes-Iturbide *et al*., 2022; Singh *et al*., 2023). The blood samples with the anticoagulant K2 EDTA were analyzed directly upon arrival at the laboratory for white blood cell (WBC), lymphocyte (LY), monocyte (MO), granulocyte (GR), red blood cell (RBC), hemoglobin (HGB) and hematocrit (HCT) analysis using the Cell-Dyn 3700 Autoanalyzer (Abbott Diagnostics, Illinois, USA).

Other blood samples without the anticoagulant K2 EDTA were centrifuged at 2000 rpm for 15 minutes to obtain separated serum and then stored in Eppendorf tubes at -20°C until further analysis. The concentrations of serum biochemical parameters such as total protein (TP), albumin (ALB), globulin (GLOB), glucose (GLU), cholesterol (CH), high-density lipoprotein (HDL), triglycerides (TRIG), urea (UR), creatinine (CREA), aspartate transferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were analyzed using commercial kits (Quimica Clinica Aplicada, Spain) with a spectrophotometric analyzer (Chem Well, Palm City, USA). In addition, the ratio of ALB to GLOB and low-density lipoprotein (LDL) were calculated according to the previously described method (Cleef *et al*., 2023).

Apparent digestibility trial

At the end of the experimental period, one lamb per replicate (eight lambs per experimental feed) was randomly selected for a nutrient digestibility trial in cages (1.40×0.5 m) equipped with an automatic feeder, a drinker and a tray for fecal sampling. Feed intake and fecal excretion were recorded daily for five days after the lambs had been acclimatized for four days and then stored at -20°C. Thereafter, 50 g of both samples were collected daily and mixed for chemical analysis of nutrient composition such as dry matter, crude protein, crude fat, ash, neutral and acid detergent fibers according to the Association of Official Analytical Chemists (AOAC, 2012) method. However, for each nutrient, feed intake and fecal excretion were used to calculate the daily intake for each nutrient (Chethan Costa *et al*., 2022). Apparent digestibility [(the difference between intake and fecal excretion for each nutrient multiplied by 100) divided by nutrient intake] was calculated according to Li *et al*. (2021).

Rumen fermentation characteristics

Rumen fluid samples (40 ml) were collected from eight lambs per experimental diet on ice in sterile tubes via a stomach tube (Anscitech, Co. Ltd., Wuhan, China) immediately after lambs were slaughteredslaughter of the lambs. The pH of each sample was measured immediately using a digital liquid pH meter (Hanna, Model 211, RI, USA). Gas production including methane, carbon dioxide and hydrogen was estimated by incubating the rumen fluid (20 mL rumen fluid and 80 mL buffer medium) with the experimental feeds at 39°C for 24 hours. The gasses were collected in an evacuated tube and analyzed by gas chromatography-mass spectrometry (GC-MS; Agilent, Palo Alto, CA, USA) according to the described method described by Lind *et al*. (2020).

All samples were centrifuged (12,000 rpm for 15 min), the supernatant part for the determination of ammonia-nitrogen and volatile fatty acids, the solid part for the estimation of bacterial quantification and then stored at -20°C. Ammonia-nitrogen was analyzed using a colorimetric spectrophotometer (Perkin Elmer, Waltham, MA, USA) according to the procedures of the Association of Official Analytical Chemists (AOAC, 2012). 5 mL of the rumen fluid supernatant was mixed with 1.5 mL of meta-phosphoric acid (25 g/100 mL) and centrifuged (10,000 rpm at 5°C for 20 min). The concentration of volatile fatty acids was analyzed by GC-MS (Agilent, Palo Alto, CA, USA) with internal standard (Augsburg, Germany) according to the previously described method (Li *et al*., 2023). The concentrations of lactate, acetate, propionate and butyrate were expressed as a percentage of total volatile fatty acids.

Total DNA was extracted from each rumen fluid solid using a commercial kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. The purity and concentration of extracted DNA was determined using the Nanodrop (Thermo, Model 2000, MA, USA). The extracted DNA were amplified by real-time quantitative polymerase chain reaction (RT-PCR system, Applied Biosystems, USA) using the bacterial target primers such as: Fibrobacter succinogenes (F:5′-GTTCGGAATTACTGGCGTAAA-3′; R:5′-CGCCTGCCTCTGAACTATC-3′). Ruminococcus albus (F:5′-CCCTAAAAGCAGTCTTAGTTCG-3′;R:5′-CCTCCTTGCGTTTAGAACA-3′). Ruminococcus flavefaciens (F:5′-ATTGTCCCAGTTCAGATTGC-3′; R:5′-GGCGTCCTC ATTGCTGTTAG-3′). Streptococcus bovis (F:5′-ATTCTT AGA CTCTT-3′; R:5′-ACCTTA TGATGGCAACTAACAATA-3′).

Butyrivbrio fibrisolvens (F:5′- CTC-3′; R:5′-CCTCC CGACA CCTATTATTCATCG-3′). general bacteria (F:5′-CGGCAACG AGCGCGAACCC-3′; R:5′-CCATTGTAGCAC GTGTGTAGCC-3′). According to the previous studies (Mirzaei-Alamouti *et al*., 2021b; Tian *et al*., 2023). Quantification of rumen bacteria (Log_{10} microbial per 1 mL of rumen fluid) was determined using a standard curve.

Rumen morphology measurements

The measurements of rumen tissue morphology such as papilla height and, width, papilla surface area, density, total surface area and stratum corneum were performed according to the method previously described previously method (Li *et al*., 2021; Han *et al*., 2022). Briefly, rumen tissue samples (3 cm^2 ventral rumen sac of eight lambs per experimental feed) were collected immediately after lambs were slaughtered. All rumen tissues were fixed in 10% formalin, dehydrated in ethanol solution (50-100%) and rinsed with xylene (Tissue-Tek VIP 5 Jr., Sakura, Japan). During preparation, the tissue was immersed in paraffin and cut (5 µm) using a microtome (Leica Biosystems, Germany), stained with hematoxylin and eosin and examined at 10 points using a microscope (Olympus, Tokyo, Japan) and image analysis software (Leica, Cambridge, UK).

Statistical data analysis

The data was were subjected to an analysis of variance (ANOVA) for each item using the Statistical Analysis system System 9.4 software (SAS, 2008) after homogeneity and normality of variances (Shapiro-Wilk and Levene tests) had beenwere checked. The mean values of the experimental feed for each item were compared using the Duncan's multiple range test at $P<0.05$. The standard error of the mean (± SEM) was reported next to the results expressed as mean values.

RESULTS AND DISCUSSION

Growth performance indicators

The effects of the experimental feeds with different levels of neutral detergent fibers on the growth performance of the lambs are shown in Table 2. No significant differences in BW were observed between the experimental feeds at 1, 28 and 56 days ($P>0.05$). In contrast, the experimental feed with a medium content of neutral detergent fiber (280 g/kg) had the highest BW at 84 days compared to the other feeds ($P<0.05$). In addition, TWG and DWG were higher in lambs fed a feed with a medium level of neutral detergent fiber than in lambs fed a high level of neutral detergent fiber from day 1 to 28, a low level of neutral detergent fiber from day

Table 2: The effects of experimental feeds with varied neutral detergent fiber levels on growth performance in growing lambs.

Items[1]	Experimental feeds[2]			SEM[3]	*P*-value
	Low	Medium	High		
From 1 to 28 days					
BW, kg, 1 day	23.71	23.61	23.51	0.301	0.894
BW, kg, 28 days	29.94	30.69	28.95	0.71	0.187
TWG, kg	6.24^{ab}	7.10^{a}	5.32^{b}	0.45	0.017
DWG, g/day	223^{ab}	253^{a}	190^{b}	14.9	0.017
DFI, g/day	1032^{c}	1246^{a}	1093^{b}	8.48	<.0001
FCR, g/g	5.20	5.08	5.50	1.18	0.962
From 29 to 56 days					
BW, kg, 56 days	36.42	37.60	35.75	0.84	0.241
TWG, kg	6.25	6.92	6.92	0.34	0.283
DWG, g/day	223	247	247	11.5	0.283
DFI, g/day	1348^{b}	1433^{a}	1498^{a}	20.2	<.0001
FCR, g/g	6.32	6.04	6.58	0.38	0.558
From 57 to 84 days					
BW, kg, 84 days	42.81^{b}	45.76^{a}	43.28^{b}	0.75	0.010
TWG, kg	6.39^{b}	8.16^{a}	7.53^{ab}	0.40	0.008
DWG, g/day	228^{b}	291^{a}	269^{ab}	13.5	0.008
DFI, g/day	1557^{b}	1702^{a}	1713^{a}	30.2	0.0006
FCR, g/g	7.40^{a}	6.00^{b}	6.83^{ab}	0.39	0.039
From 1 to 84 days					
TWG, kg	19.03^{b}	22.17^{a}	19.77^{b}	0.51	<.0001
DWG, g/day	226^{b}	264^{a}	235^{b}	6.10	<.0001
DFI, g/day	1312^{b}	1461^{a}	1434^{a}	15.2	<.0001
FCR, g/g	5.89^{ab}	5.60^{b}	6.18^{a}	0.16	0.026

$^{a-b}$ Superscript letters indicate significant differences ($p<0.05$) between the experimental feeds with different levels of neutral detergent fiber within a row. [1]BW= Body weight, TWG= Total weight gain, DWG= Daily weight gain, DFI= Daily feed intake and FCR= Feed conversion ratio. [2] The experimental feeds: low = 210 g, medium = 280 g and high = 350 g of neutral detergent fibers per kg of feed.[3] SEM = Standard error of means.

57 to 84 and other experimental feeds from day 1 to 84 (P<0.05). No significant differences in TWG and DWG were observed among experimental feeds from day 29 to 56 (P>0.05). Lambs fed medium and high levels of neutral detergent fiber had the highest DFI compared to lambs fed low levels of neutral detergent fiber from day 1 to 28, from day 29 to 56, from day 57 to 84, and overall (P<0.05). In lambs fed a medium level of neutral detergent fiber, FCR improved compared to the low level from day 57 to 84 (P<0.05) and the high level from day 1 to 84 (P< 0.05), but was not affected by other levels. Whereas, no significant differences in FCR was observed among experimental feeds from day 1 to 28 and day 29 to 56 (P>0.05).

Hema-biochemical parameters

The effects of the experimental feeds with different levels of neutral detergent fibers on the hematological and biochemical parameters of the lambs are shown in Table 3. Hematological parameters, including WBC count, decreased while RBC count and HGB increased in lambs fed medium level of neutral detergent fiber compared to the other feeds (P<0.05). In contrast, no significant differences in other hematology parameters (LY, MO, GR and HCT) were observed among experimental feeds (P>0.05). In addition, blood biochemical parameters such as TP and GLU concentrations increased in lambs fed medium and high levels of neutral detergent fiber compared to lambs fed low level of neutral detergent fiber (P<0.05). Concentration of GLOB increased and ALB/Glob ratio decreased in lambs fed a medium level of neutral detergent fiber compared to lambs fed a low level of neutral detergent fiber (P<0.05), but did not affected with lambs fed high level of neutral detergent fiber. In contrast, other blood biochemical parameters did not any affected by experimental feeds with different levels of neutral detergent fibers (P>0.05).

Daily intake and apparent digestibility of nutrient

The effects of the experimental feeds with different levels of neutral detergent fiber on the daily intake and apparent digestibility of nutrients in lambs are shown in Table 4. Dry matter, organic matter, neutral detergent fiber and acid detergent fiber increased (P<0.05) in lambs fed experimental

Table 3: The effects of experimental feeds with varied neutral detergent fiber levels on hema-biochemical parameters in growing lambs.

Items[1]	Experimental feeds[2]			SEM[3]	*P*-value
	Low	Medium	High		
Hematology parameters					
WBC (10^g/l)	13.34[a]	9.80[b]	13.23[a]	1.05	0.037
LY (%)	37.97	35.54	39.37	2.88	0.641
MO (%)	5.57	5.71	5.59	0.61	0.984
GR (%)	56.47	58.75	55.04	2.78	0.640
RBC (10^12/l)	12.39[b]	13.10[a]	12.48[b]	0.18	0.032
HGB (g/dl)	9.30[b]	11.57[a]	9.70[b]	0.19	<.0001
HCT (%)	28.05	30.09	29.52	0.60	0.062
Blood biochemical parameters					
TP (g/dl)	5.26[b]	6.39[a]	5.97[a]	0.16	0.0001
ALB (g/dl)	3.39	3.42	3.52	0.16	0.841
GLOB (g/dl)	1.87[b]	2.96[a]	2.45[ab]	0.19	0.001
ALB/Glob ratio	2.13[a]	1.30[b]	1.50[ab]	0.22	0.035
GLU (mg/dl)	63.51[b]	77.92[a]	72.42[a]	2.07	0.0001
CHOL (mg/dl)	80.44	82.97	87.51	3.63	0.390
HDL (mg/dl)	35.88	39.50	38.64	2.16	0.472
LDL (mg/dl)	24.53	24.70	28.94	2.19	0.286
TRIG (mg/dl)	100.16	93.90	99.65	5.76	0.698
UREA (mg/dl)	15.72	17.20	15.59	1.66	0.751
CREA (mg/dl)	1.155	1.201	1.130	0.11	0.903
AST (U/L)	86	76	82	4.30	0.328
ALT (U/L)	20	18	20	1.23	0.424
ALP (U/L)	341	296	314	18.5	0.231

[a-b]Superscript letters indicate significant differences (p<0.05) between the experimental feeds with different levels of neutral detergent fiber within a row.

[1]WBC= White blood cells, LY= Lymphocytes, MO= Monocytes, GR= Granulocytes, RBC= Red blood cells, HGB= Hemoglobin, HCT= Hematocrit, TP= total protein, ALB= albumin, GLOB= globulin, GLU= glucose, CH= cholesterol, HDL= high-density lipoprotein, TRIG= triglycerides, UR= urea, CREA= Creatinine, AST= Aspartate transferase, ALT= Alanine transaminase and ALP= Alkaline phosphatase.

[2]The experimental feeds: low = 210 g, medium = 280 g and high = 350 g of neutral detergent fibers per kg of feed.

[3]SEM= Standard error of means.

Table 4: The effects of experimental feeds with varied neutral detergent fiber levels on daily intake and apparent digestibility of nutrient in growing lambs.

Items	Experimental feeds[1]			SEM[2]	*P*-value
	Low	Medium	High		
Daily intake of nutrients (g/day)					
Dry matter	1266[b]	1424[a]	1358[a]	39.1	0.031
Organic matter	1141[b]	1301[a]	1244[a]	25.3	0.017
Crude protein	163[b]	223[a]	172[b]	12.1	0.013
Crude fat	48	57	52	3.60	0.337
Ash	121	122	115	8.82	0.823
Neutral detergent fiber	266[b]	402[a]	446[a]	22.5	0.0008
Acid detergent fiber	175[b]	229[a]	231[a]	13.7	0.029
Apparent digestibility of nutrient (%)					
Dry matter	72.71[b]	82.70[a]	72.29[b]	1.31	0.0005
Organic matter	83.82[b]	90.59[a]	83.04[b]	0.92	0.0005
Crude protein	69.03[b]	79.78[a]	65.72[b]	3.46	0.044
Crude fat	87.50	90.41	86.15	2.11	0.385
Ash	34.79	53.41	29.75	6.75	0.079
Neutral detergent fiber	37.99[c]	70.87[a]	63.58[b]	4.65	0.001
Acid detergent fiber	45.68[c]	72.28[a]	56.23[b]	4.86	0.011

[a-b]Superscript letters indicate significant differences (p<0.05) between the experimental feeds with different levels of neutral detergent fiber within a row.

[1]The experimental feeds: low = 210 g, medium = 280 g and high = 350 g of neutral detergent fibers per kg of feed.

[2]SEM= Standard error of means.

Table 5: The effects of experimental feeds with varied neutral detergent fiber levels on rumen fermentation characteristics in growing lambs.

Items	Experimental feeds[1]			SEM[2]	*P*-value
	Low	Medium	High		
PH	6.19[a]	5.72[b]	5.72[b]	0.06	<.0001
Ammonia nitrogen (mM)	7.89	8.37	7.49	0.58	0.595
Gas production					
Methane gas (ml)	41.40[a]	35.20[b]	41.47[a]	0.76	0.001
Carbon dioxide gas (ml)	165.14[a]	133.95[b]	163.58[a]	1.98	<.0001
Hydrogen gas (ml)	1.99[c]	2.32[b]	2.86[a]	0.04	<.0001
Total gas (ml)	208.53[a]	171.47[b]	207.92[a]	2.49	<.0001
Volatile fatty acids concentration					
Lactate (%)	27.52[b]	43.42[a]	28.69[b]	3.28	0.007
Acetate (%)	31.64[b]	33.09[b]	38.14[a]	1.27	0.006
Propionate (%)	17.60[c]	27.89[a]	21.22[b]	1.73	0.004
Butyrate (%)	7.34[b]	11.50[a]	11.95[a]	0.79	0.002
Acetate / propionate ratio	1.72[a]	1.13[b]	1.80[a]	0.11	0.003
Total volatile fatty acids (mg/ml)	7.97[b]	10.25[a]	8.93[b]	0.29	0.0005
Ruminal bacterial quantification					
Fibrobacter succinogenes	3.98[b]	5.40[a]	4.16[b]	0.32	0.006
Ruminococcus albus	4.72[b]	5.89[a]	5.03[b]	0.24	0.003
Ruminococcus flavefaciens	6.34[b]	7.97[a]	6.50[b]	0.39	0.008
Streptococcus bovis	6.09	6.61	6.13	0.28	0.370
Butyrivbrio fibrisolvens	4.93	6.22	5.70	0.40	0.092
General bacteria	6.41[b]	7.45[a]	6.64[b]	0.20	0.002

[a-b]Superscript letters indicate significant differences (p<0.05) between the experimental feeds with different levels of neutral detergent fiber within a row.

[1]The experimental feeds: low = 210 g, medium = 280 g and high = 350 g of neutral detergent fibers per kg of feed.

[2]SEM = Standard error of means.

[3]Log10 microbial per 1 mL of rumen fluid.

feeds with medium and high level of neutral detergent fiber compared to lambs fed experimental feeds with low level of neutral detergent fiber. In addition, lambs fed a medium level of neutral detergent fiber had the highest crude protein content compared to the other experimental feeds (P<0.05). In contrast, crude fat and ash contents did not any affected by experimental feeds with different levels of neutral detergent fibers (P>0.05).

The digestibility of dry matter, organic matter and crude protein were higher (P<0.05) in lambs fed a medium level of neutral detergent fiber compared to the other experimental feeds. In addition, the digestibility of neutral and acid detergent fiber was higher in lambs fed a medium and then a high level of neutral detergent fiber than in lambs fed a low level of neutral detergent fiber (P<0.05). In contrast, apparent digestibility of crude fat and ash did not any affected by experimental feeds with different levels of neutral detergent fibers (P>0.05).

Rumen fermentation characteristics

The effects of the experimental feeds with different levels of neutral detergent fibers on the fermentation characteristics in rumen of the lambs are shown in Table 5. Rumen pH decreased in lambs fed medium and high level of neutral detergent fiber compared to low level of neutral detergent fiber (P<0.05). Whereas, Ammonia nitrogen concentration did not any affected by experimental feeds with different levels of neutral detergent fibers (P>0.05).

The production of gasses in the rumen such as methane, carbon dioxide and total gas was lowest in lambs fed a medium level of neutral detergent fiber compared to the experimental feeds with low and high levels of neutral detergent fiber (P<0.05). In contrast, lambs fed a high and then a medium level of neutral detergent fiber had an increased concentration of hydrogen gas compared to lambs fed a low level of neutral detergent fiber (P<0.05).

The concentration of lactate and total volatile fatty acids were highest in lambs fed feed with a medium level of neutral detergent fiber compared to experimental feeds with a low and high level of neutral detergent fiber (P<0.05). In addition, propionate and butyrate concentrations were higher in lambs fed feed with medium and high level of neutral detergent fiber compared to lambs fed feed with low level of neutral detergent fiber (P<0.05). The acetate concentration was higher in lambs fed high level of neutral detergent fiber than in the other experimental feeds (P<0.05). The acetate/propionate ratio was lower in lambs fed a medium level of neutral detergent fiber than in the other experimental feeds (P<0.05).

The quantify of *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and general bacteria were higher in lambs fed a medium level of neutral detergent fiber than in the other experimental feeds (P<0.05). In contrast, *Streptococcus bovis* and *Butyrivbrio fibrisolven* did not any affected by experimental feeds with different levels of neutral detergent fibers (P>0.05).

Rumen morphology measurements

The effects of the experimental feeds with different levels of neutral detergent fibers on the measurements of the morphology of the lambs' rumen tissue are shown in Table 6. Papilla height, papilla surface area, total papilla, surface area and corneum thickness were increased in lambs fed medium and high level of neutral detergent fiber compared to the experimental feed with low neutral detergent fibers (P<0.05). In contrast, papilla width, density of papilla did not any affected by experimental feeds with different levels of neutral detergent fibers (P>0.05).

Neutral detergent fibers have been considered an essential component of lamb feed as they influence DFI, digestibility and microbial activity in the rumen and thus growth (Grabber *et al*., 2009; Carrillo-Díaz *et al*., 2022). The results of this trial indicate that feeding lambs with a medium level of neutral detergent fiber (280 g/kg) could be optimal for lamb growth performance, especially in terms of BW, DWG and TWG with improved FCR. This result is consistent with Xie *et al*. (2020), who reported improved growth performance in lambs fed 220 and 260 g neutral detergent fibers per kg of feed. In contrast, feeding a high level of neutral detergent fiber level (559.3 g/kg) as roughage in the total mixed ratio had a negative effect on DWG and FCR (Blanco *et al*., 2015). Ozdogan *et al*. (2017) found that neutral detergent fiber levels of 271 and 241 g/kg improved FCR and had no effect on DMI and DWG of

Table 6: The effects of experimental feeds with varied neutral detergent fiber levels on rumen tissue morphology measurements in growing lambs.

Items	Experimental feeds[1]			SEM[2]	*P*-value
	Low	Medium	High		
Papilla height (mm)	1.48[b]	2.04[a]	1.87[a]	0.09	0.0008
Papilla width (mm)	0.29	0.32	0.33	0.01	0.138
Surface area of papilla (mm^2)	1.36[b]	2.04[a]	1.94[a]	0.11	0.0004
Density of papilla (n/cm^2)	71	69	69	3.14	0.828
Total surface of papillae (mm^2/cm^2)	60.54[b]	88.34[a]	84.08[a]	5.21	0.001
Corneum thickness (mm)	0.027[b]	0.039[a]	0.046[a]	0.002	<.0001

[a-b] Superscript letters indicate significant differences (p<0.05) between the experimental feeds with different levels of neutral detergent fiber within a row. [1]The experimental feeds: low = 210 g, medium = 280 g and high = 350 g of neutral detergent fibers per kg of feed. [2]SEM = Standard error of means.

lambs. Lambs fed medium (280 g/kg) and high (350 g/kg) levels of neutral detergent fiber had higher DFI, suggesting that these experimental feeds stimulated appetite and improved nutrient utilization. The study by Gallo *et al*. (2019) showed that a neutral detergent fiber level of 250 g/kg reduced DFI in lambs, which could be due to rumen development and increased rumination and fermentation activity. However, lambs fed a low level of neutral detergent fiber (210 g/kg) in this study had lower DFI, which could be due to lower digestibility.

Haematological and biochemical parameters provide important information about the general health, nutritional status and metabolic processes in growing lambs (Lotfollahzadeh *et al*., 2016). The results obtained show a decrease in WBC count and an increase in RBC count and HGB in lambs fed a medium level of neutral detergent fiber. These results could indicate an improvement in the general health and oxygen carrying capacity of these lambs, which could be associated with improved metabolic efficiency and growth performance. Some studies have reported that feeds high in neutral detergent fiber can lead to increased inflammatory responses and oxidative stress (Li *et al*., 2024), which could be reflected in changes in WBC counts. Therefore, the observed decrease in WBC count in lambs fed a medium level of neutral detergent fiber could indicate a decrease in inflammatory markers. The increased TP and GLU concentrations in lambs fed medium and high levels of neutral detergent fiber indicate an improvement in protein and energy metabolism due to the increased DFI and daily intake of nutrients such as dry matter, organic matter, crude protein, neutral detergent fiber and acid detergent fiber. Gallo *et al*. (2019) reported that feeding neutral detergent fiber to lambs had no effect on haematological or biochemical parameters. In contrast, Zhao *et al*. (2015) reported that lower levels of neutral detergent fiber increased glucose concentration in dairy cows. The higher GLOB concentration and lower ALB/GLOB ratio in lambs fed a medium level of neutral detergent fiber could indicate an improved immune response or increased liver activity. The improved hematological and biochemical parameters could have a positive effect on the health of lambs fed a medium level of neutral detergent fiber.

In the present study, the digestibility of nutrients, including dry matter, organic matter, crude protein, neutral detergent fiber and acid detergent fiber, was improved in lambs fed a medium level of neutral detergent fiber (280 g/kg). This indicates that rumen fermentation characteristics and nutrient utilization were improved by this experimental diet. Previous studies have shown that optimal levels of neutral detergent fiber can influence rumen fermentation characteristics including microbial activity and fiber digestion (Chen *et al*., 2024). However, a medium level of neutral detergent fiber can create an optimal environment for fiber-digesting microorganisms, resulting in improved nutrient digestibility in lambs. In addition, the increased abundance of *Fibrobacter succinogenes*, *Ruminococcus albus, Ruminococcus flavefaciens* and general bacteria in the medium level of neutral detergent fiber indicates a favorable environment for fiber-digesting microorganisms. This is consistent with a study that found that feeding lambs high levels of neutral detergent fiber stimulated cellulose-degrading bacteria, resulting in increased acetate concentrations, while low levels stimulated amylolytic bacteria and increased propionate concentrations (Holtzapple *et al*., 2022). In addition, lambs receiving 250 g neutral detergent fiber/kg feed can improve fiber digestibility due to increased microbial activity (Asadollahi *et al*., 2017). Higher concentrations of total volatile fatty acids, lactate, propionate and butyrate in lambs fed a medium level of neutral detergent fiber resulted in increased rumen fermentation activity. This confirms our findings that feeding lambs 280 g of neutral detergent fiber may improve microbial function and nutrient utilization in lambs. Previous studies have shown that neutral detergent fibers can stimulate rumen microbial activity and volatile fatty acid production (Wang *et al*., 2020). The higher acetate concentration in lambs fed a high level of neutral detergent fiber indicates a shift towards a more fibrous fermentation pattern and could therefore be related to increased rumen fiber availability. In addition, rumen pH was lower in lambs fed medium and high levels of neutral detergent fiber. Rumen pH is primarily determined by the balance between buffer secretion and VFA synthesis, which is the state of natural rumen processes (Chen *et al*., 2024). Higher neutral detergent fibers content leads to increased saliva production, which acts as a natural buffer (Hyder *et al*., 2017). The lack of an effect of neutral detergent fiber levels on ammonia-nitrogen concentration is unexpected, as higher levels of neutral detergent fiber lead to increased protein degradation and ammonia production (Manatbay *et al*., 2014). However, the results suggest that the factors influencing ammonia production and utilization were balanced in this experiment. In contrast, Ma *et al*. (2015) reported that ammonia-nitrogen concentration increased with higher fermentation processes and neutral detergent fiber intake. It is interesting to note the lower production of methane, carbon dioxide and total gas in lambs that received a medium level of neutral detergent fiber. This result suggests a possible reduction in total gas production, which could have a positive impact on both lamb performance and environmental impact (Wang *et al*., 2019). The rumen plays a crucial role in ruminant digestion and is therefore directly related to functional capacity (Plaizier *et al*., 2022). The data on rumen morphology showed that medium and high levels of neutral detergent fiber promoted rumen development and led to an increase in papilla height, surface area and total papilla area. This indicates improved rumen capacity and nutrient intake. The increased rumen surface area provides a larger area for microbial attachment and fermentation, leading to improved nutrient utilization. This is consistent with a previous study which found that lambs fed optimal levels of dietary fiber had improved rumen digestibility, metabolic and morphological

characteristics (Gallo *et al*. 2014). According to previous studies, roughages with a high content of neutral detergent fiber support the rumen epithelium and papillae, motility, size and muscle development and promote rumination (van Ackeren *et al*., 2009; Alvarez-Rodriguez *et al*., 2012).

CONCLUSION

In conclusion, the findings of this study indicate that a medium level of neutral detergent fiber (280 g /kg) in the experimental feed improved growth performance parameters and some blood parameters. Additionally, this experimental feed showed higher apparent digestibility of nutrients except crude fat and ash as well as enhanced rumen fermentation characteristics, such as increased volatile fatty acid production and microbial abundance. Rumen morphology was also positively influenced by the medium neutral detergent fiber level, with higher papillae height and increased surface area. These findings suggest that optimizing neutral detergent fiber intake (280 g/kg) is crucial for maximizing growth performance and overall well-being in growing lambs.

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Disclaimers

The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Animal care, design, and sampling were performed in accordance with the Research Ethics Committee of King Saud University, Riyadh, Saudi Arabia (No.: KSU-SE-22-26).

Conflict of interest

All authors declare no conflicts of interest among them.

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