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Effect of Different Dietary Buffer Sources and Roughage-to-Concentrate Ratios on Growth Performance, Rumen Fermentation, and Health Status of Growing Lambs

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ABSTRACT

A total of 90 Barki lambs (average body weight: 24.43 kg) were randomly and equally allotted into nine groups to assess the role of dietary buffer addition on growth performance, rumen fermentation, and overall health status. The lambs were fed diets with different roughage-to-concentrate (R:C) ratios: 60:40, 40:60, and 20:80. Within each R:C ratio, three dietary treatments were applied: no buffer (control), sodium bicarbonate, and sodium bentonite supplementation. Groups 1, 2, and 3 were fed the 60R:40C diet with no buffer, sodium bicarbonate, and sodium bentonite, respectively. Groups 4, 5, and 6 received the 40R:60C diet with the same buffer treatments, and Groups 7, 8, and 9 were fed the 20R:80C diet under the same supplementation conditions. Results showed that, within the 60R:40C group, both buffers improved ($P \geq 0.05$) body weight (BW), total gain (TG), total feed intake (TFI), and feed conversion ratio (FCR), while significantly ($P<0.05$) increasing rumen total volatile fatty acids (TVFAs), intestinal villi length, phagocytic activity, and serum bactericidal activity. These treatments also significantly reduced serum lysozyme activity and rumen ammonia nitrogen (NH3-N) levels. In the absence of buffer supplementation, the 40R:60C diet resulted in improved BW, TG, intestinal villi length, and reduced TFI, leading to improved FCR. It also enhanced phagocytic and bactericidal activities and lowered lysozyme levels. This ratio did not significantly affect rumen pH. However, the 20R:80C diet significantly reduced rumen pH. Supplementation of buffers in the 20R:80C group improved BW, TG, villi length, TVFAs, and concentrations of acetic and propionic acids. It also reduced rumen NH_3-N and mitigated the negative impacts of high concentrate feeding on rumen pH while lowering TFI and improving FCR. Overall, the 40R:60C ratio supplemented with sodium bicarbonate yielded the most favorable outcomes in terms of growth performance and rumen health. Therefore, we recommend this combination for optimal productivity in growing lambs.

Keywords: Concentrate, Growth, Rumen health, Sodium bentonite, Sodium bicarbonate.

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INTRODUCTION

The rumen microflora plays a critical role in carbohydrate, starch, sugar, proteins and fat digestion through anaerobic fermentation that yields volatile fatty acids (propionic, acetic and butyric acid) and microbial proteins, which supply the ruminants with the energy and proteins required for growth and production (**Ceconi *et al.*, 2015; Jiang *et al.*, 2015**). The nutrition is a major factor influencing the ruminants microflora and immunity, thereby affecting their growth and productive performance (**Clark, 1975**). The concentrate supplementation between 60 and 80% is associated with digestibility and performance enhancement of crossbred lambs without clear feed intake or ingestive behavior (**Parente *et al.*, 2016**). Meanwhile, the inclusion of concentrate in the feed altered the rumen fermentation to propionate rather than acetate, thus enhancing Tibetan sheep efficiency of energy utilization (**Liu *et al.*, 2019**).

Unless the buffering capacity of the rumen is able to keep pace with acid accumulation, low milk fat, rumen acidosis, laminitis, inflammation and diarrhoea will be the results (**Enemark 2008**). **Aikman *et al.,* (2011)** clarified that lactic acid reduced the ruminal pH, thus resulting in digestive disorders and decreasing the animal's performance. Buffers can improve the ruminal environmental conditions through modulating the ruminal contents acidity and preventing the severe pH drops **(Le Ruyet and Tucker, 1992).** Water passage through the reticulo-rumen is a vital digestion component that acts as a digesta vehicle to transport it to the lower gut. The increased dilution rate of the liquid phase influences the rumen microbes and their activities (**Van Soest, 1994**). The higher the dietary concentrate levels, the slower rate of rumen liquid dilution **(Goetsch and Galyean, 1982).** Researchers have focused on the buffering supplement's ability to increase the rumen liquid dilution rate and protein flow to the small intestine (**Nangia and Sharma, 1994).**

Using different dietary buffering agents could contribute to controlling rumen fermentation, hence enhancing rumen pH, feed efficiency, and carcass characteristics, coupled with lower growing Awassi lambs body fat contents (**Alhidary *et al.,* 2019**). Various buffers, such as sesquisodium carbonate, sodium bicarbonate, sodium bentonite and AcidBuf, have been included in the diets of ruminants for production improvement (**Cruywagen *et al.,* 2015; Alhidary *et al.,* 2019; Asadi *et al.,* 2024**). The dietary inclusion of sodium bicarbonate helps in maintaining the pH of the rumen in dairy cows (**West *et al.* 1987**), buffaloes (**Koul *et al.* 1998**) and lambs (**Santra *et al.,* 2003**) and is also associated with a higher dry matter intake and body weight gain **(Tripathi *et al.,* 2004).** Sodium bentonite supplementation improves rumen pH and ruminal microorganisms' metabolic activity; therefore, it enhances lambs' growth performance and feed efficiency (**Aitchison *et al.,* 1986; Aydin *et al.,* 2020**). There hasn't been much available information considering the sodium bentonite inclusion as a buffer and its comparable impact with sodium bicarbonate in lambs rations containing different levels of concentrate.

Therefore, this study aimed to evaluate the comparative efficacy of sodium bicarbonate and sodium bentonite supplementation on rumen fermentation and its subsequent effects on growth performance and health status of growing lambs fed on different roughage concentrate ratios.

MATERIALS AND METHODS

Experimental animals used

All animals` management procedures were done according to the Institutional Animal Care and Use Committee of the University of Alexandria (ALEXU-IACUC-013-2020-12-13-MD (2)-66) approved protocol. A total of 90 Barki lambs (average BW, 24.43±0.92 kg) of the same age (average 8 months) were randomly assigned to nine groups, with 10 lambs per group, assigned to equal-sized pens (2*2 m). At the time of arrival, lambs were individually weighed, ear-tagged, and vaccinated for viral and clostridial diseases. Lambs were dewormed with Ivomec Plus and s/c injection of Exceed for protection against respiratory pathogens.

Experimental design and feeding program

The first two weeks were for adaptation. Lambs in the first treatment (T1) were fed on a 60:40 roughage:concentrate (R:C) ratio with no buffer; T2 were fed on a 60R:40C ratio with sodium bicarbonate (BESIN TURU FOOD GRADE); and T3, 60R:40C with sodium bentonite (Nutrimax Egypt for Animal Health) supplementation. Each buffer was added at 1% of the ration (as recommended in the manufacturers' instructions) instead of corn. T4, T5 and T6 were fed on a 40R:60C ratio with no buffer, sodium bicarbonate and odium bentonite respectively, and the last three treatments (T7, T8 and T9) were fed on the same previous buffer line with a 20R:80C ratio. The **NRC (2007)** was followed in the formulation of different diets. Lambs were fed twice daily, and the actual amount of feed consumed was measured daily by weighing the feed delivered and subtracting the refusals left. The experiment lasted for 3 months. At the end of the trial, blood (from the jugular vein in the neck) and rumen contents (using a stomach tube) and intestine (after slaughtering) samples were collected from three lambs per group, as well as collecting samples from diets for later analysis.

Chemical analysis

Feed samples analysis

DM, ash, organic matter, crude protein and ether extract contents in feed samples were determined according to **AOAC (2005).** According to **Van Soest *et al.,* (1991),** the neutral detergent fiber (NDF) and acid detergent fiber (ADF) were identified. Analysis of NDF were performed by heat-stable α-amylase using and expressed exclusive of residual ash (aNDFom) using an ANKOM 200 Fiber Analyzer unit (ANKOM Technology Crop., Macedon, NY, USA). The ADF was expressed exclusive of residual ash (ADFom). **Van Soest (1973)** method was used for lignin % detection, the cellulose (cellulose, g / kg DM = ADFom – lignin) and hemi-cellulose (hemicellulose, g / kg DM = aNDFom – ADFom) contents were calculated**.** According to **Slavin (1968)** and **Gericke and Kurmies (1952),** flame photometer and colorimetric procedures were used for Ca and P assesment, respectively in the ground well prepared feed samples **(Table 1).**

Table 1: Ingredient and chemical composition of the used experimental diets.

	Roughage: Concentrate ratio		
Ingredients	60:40	40:60	20:80
Berseem hay	60	40	20
Yellow corn	12.5	23.8	28.35
Soybean oil meal (46%)	13.65	15.6	16
Wheat bran	8.4	15.5	30
Ebilac -fort dry fat[a]	1.5	0.5	0
Limestone	0.1	0.75	1.8
Molasses	3	3	3
Mg oxide	0.1	0.1	0.1
Premix[b]	0.3	0.3	0.3
Salt	0.25	0.25	0.25
Anti mycotoxin[c]	0.1	0.1	0.1
Yeast[d]	0.1	0.1	0.1
Total	100	100	100
	Chemical composition		
Moisture %	10.98	11.09	10.48
Crude Protein	15.76	15.55	15.43
Ether Extract	2.24	3.12	3.54
Organic matter	87.34	90.44	92.56
Neutral detregent fiber (NDF)	51.55	48.42	45.75
Acid detregent fiber (ADF)	19.75	20.35	21.51
Acid detregent lignin (ADL)	10.74	8.56	6.84
Hemicellulose	31.8	28.07	24.24
Cellulose	10.34	11.55	16.48
Calcium	1.12	0.92	0.91
Phosphoruse	0.38	0.43	0.46
TDN**	69.4	72.2	73.9

[a]Ebilac-fort® locally produced contain dry fat 84.85% (palm oil 37%, soybean oil 37% and linseed oil 10.85%), calcium oxide 15%, antioxidant (6443) 0.15% and moisture 4%. [b]Each 3 kg contains: Vit A (10000000IU), Vit D (2000000IU), Vit E(10g), Iron (50g), Copper (8g), Zinc (30g), Manganese (40g), Iodine (0.5g), Selenium (0.1g), Cobalt (0.1g) and carrier calcium carbonate up to 3 kg**.** [c]Fer Mos®, each 1 kg contain Saccharomyces cerevisiae cell wall 500gm, Beta glucan112.5 gm, Mannan oligosaccharide (MOS) 112.5 gm and carrier silicate up to 1kg and produced by Micron Bio-Systems Company. [d]Beta Mos®, each 1 kg contain dried extract of Saccharomyces cerevisiae 1000 gm, Beta-glucan 250 gm, mannan oligosaccharide (MOS) 200 gm and produced by New Gene International Trading Company. **Total digestible neutrients, calculated according to feedstuffs composition in **NRC (2007).**

Growth performance

Using PEC scale the initial, final body weight (BW) as well as total feed intake (TFI) for each lamb was recorded. Total body weight gain (TBWG) and average feed conversion ratio (AFCR) were calculated.

Blood parameters for evaluation of immune response

Differential leucocyte counts and phagocytosis

Blood samples were taken in tubes containing anticoagulant EDTA in order to determine the differential leucocyte count in accordance with **Gross and Siegel (1983)** and the phagocytosis in accordance with **Kawahara *et al.*, (1991).**

Serum lysozyme activity and bactericidal activity

Blood samples without anticoagulant were collected and centrifuged for serum separation to measure the lysozyme activity with the turbidimetric method described by **Engstad *et al.*, (1992)** and bactericidal activity to Aeromonas hydrophila strain in accordance with **Rainger and Rowley (1993)**. The results were recorded as survival index (SI) **(Ward Law and Unlles, 1978).** The formula used to calculate the values was SI = Colony Forming Units (CFU) at Finish / CFU at Start x 100.

Determination of rumen fermentation parameters

Rumen contents samples

Samples were taken were taken using stomach tube only one time at 2 hours after feeding and acidified with 50% H2SO4 to PH 2, and then frozen at -20 ˚C for further measurement of volatile fatty acids and rumen (NH3-N).

Rumen PH

Rumen contents samples were taken four time, immedietally after feeding and every 2 hours (hrs) till 6 hrs following feeding (at 0, 2, 4 and 6 hrs following feeding). A portable pH meter (Orion research model 201) for immedietaly pH detection.

Rumen (NH_3-N)

Rumen liquid were filterd and underwent centrifugation at 12,000 xg for 20 minutes. A volume of 5 ml from the supernatant was extracted and reserved for NH_3-N levels analysis, following the methodology outlined by Parsons *et al.,* (1984).

Volatile fatty acids

Rumen-filtered specimens were thawed at 4 °C before analysis, accordance the modified protocol established of **Cottyn *et al.,* (1968).** The samples were analyzed through gas chromatography, utilizing a Flame Ionization Detector (FID) and a capillary column (DB-FFAP, 122–3232).

Determination of intestinal histopathology

During slaughtering, the intestinal tissues (duodenum) were collected, rinsed with saline and preserved in 10% formalin for no less than two days. Slides were created and stained with Hematoxylin and Eosin (H&E) for morphological evaluation of both tissues **(Bancroft and Layton, 2019).**

Statistical analysis

The collected data underwent statistical analysis using a two-way analysis of variance (ANOVA), employing Tukey's multiple comparison test using the SPSS software statistical program (SPSS for Windows ver.27, USA). Graph Pad Prism version 6.0 was used to create the graphs. The results were presented as mean ± standard error of the mean (SEM), with differences regarded as significant at P< 0.05.

RESULTS

Growth performance and feed efficiency parameters

The results in **Table 2** revealed that both buffers (sodium bicarbonate and sodium bentonite) with a 60:40 roughage concentrate (R:C) ratio non-significantly (P≥0.05) improved BW, gain, TFI and AFCR, with the better performance for bentonite. Regarding the R:C ratio with no buffer, data showed that the 40:60 ratio enhanced (P≥0.05) BW and TBWG and reduced TFI, which reflected on better FCR. A 20 R:80 C ratio had no significant effect on growth but lowered feed intake and AFCR. Using buffers with higher concentration ratios numerically increased BW and gain with lowered AFCR. A 40:60 R:C ratio with sodium bicarbonate recorded the best growth performance, followed by sodium bentonite with 40:60 and then 60:40 R:C ratios.

Table 2: Impact of buffer source on body weight development (kg/animal), feed intake (kg/animal) and feed conversion ratio of growing barki lambs fed on rations containing different roughage : concentrate ratios.

İtems / Treatments	İnitial weight (kg)	Final weight (kg)	Total gain (kg)	Total feed intake (kg)	Average feed conversion ratio
T1	24.33±1.09	41.83±2.65	17.50±1.99	116.14	6.64±0.37[a]
T2	24.33±1.06	42.50±2.67	18.17±1.81	116.55	6.41±0.29[ab]
T3	24.50±0.75	43.33±2.03	18.83±1.38	116.52	6.19±0.89[b]
T4	24.50±0.85	43.00±3.08	18.50±2.53	114.14	6.17±1.48[b]
T5	24.33±0.99	44.00±1.81	19.67±1.78	114.19	5.8±0.23[b]
T6	24.33±1.09	43.33±1.82	19.00±1.34	113.28	5.96±0.68[b]
T7	24.33±1.06	41.67±2.64	17.33±2.02	111.19	6.42±0.63[ab]
T8	24.50±0.98	42.67±0.87	18.17±0.95	113.13	6.22±0.60[b]
T9	24.67±0.80	42.33±1.56	17.67±1.12	112.07	6.34±0.20[ab]
P value					
R:C ratio	0. 966	0. 723	0.676		0.890
Buffer	0. 948	0. 945	0.924		0.010
Interaction	0.981	0.933	0.958		0.246

Values are expressed as Mean±SE. Means in the same column different superscript letter are significantly at (P<0.05). T1: R60:C40 (Without buffer). T2: R60:C40 (Sodium bicarb). T3: R60:C40 (Sodium bentonite). T4: R40: C60 (Without buffer). T5: R40: C60 (Sodium bicarb). T6 R40: C60 (Sodium bentonite). T7: R20: C80 (Without buffer). T8: R20: C80 (Sodium bicarb). T9 R80: C20 (Sodium bentonite).

Immune response

The statistical analysis of data in **Table 3** indicated a slight increase in neutrophil % and a corresponding slight decrease in lymphocyte % as the roughage % decreased from 60 to 40 and 20. As a result, the neutrophil-to-lymphocyte (N/L) ratio also declined slightly with the lower R:C ratios. Supplementation with sodium bicarbonate had no significant effect on neutrophil, lymphocyte, or N/L ratio. However, sodium bentonite had a non-significant ($P \geq 0.05$) increase in neutrophils and a decrease in lymphocytes, leading to a higher N/L ratio compared to Barki lambs fed the same diet without buffer. Regarding the R:C ratio with no buffer, data showed that a 40:60 ratio non-significantly ($P \geq 0.05$) elevated phagocytosis and bactericidal activity and reduced lysozyme. 20 R:80 C increased ($P \geq 0.05$) bactericidal and decreased lysozyme. Feeding a 40R:60C ratio with sodium bicarbonate recorded the highest bactericidal and the lowest lysozyme. This ratio with sodium bentonite recorded the highest phagocytosis.

Rumen fermentation parameters

Tables 4 showed that ruminal ammonia concentrations tended to increase as the R:C ratio decreased from R60:C40 to R40:C60 and R20:C80, although these changes were not statistically significant ($P \geq 0.05$). The lowest R:C ratio (R20:C80) exhibited a significantly higher ($P \leq 0.05$) ammonia concentration compared to those fed diets with higher roughage levels. Ruminal total volatile fatty acids (TVFAs) and propionic acid concentrations significantly increased ($P \leq 0.05$) as the R:C ratio decreased from 60: 40 to 40:60 and 20:80. The ruminal pH (table 4) significantly ($P \leq 0.05$) decreased as the R:C ratio was reduced from R60:C40 to R40:C60 or R20:C80. Moreover, it was noticed that the pH of the rumen in all experimental groups increased at 4 and 6 hours post-feeding. The addition of ruminal buffers, such as sodium bicarbonate or sodium bentonite, to the total mixed ration (TMR) of Barki lambs resulted in a non-significant increase in ruminal fluid pH compared to lambs fed the same ration without buffer supplementation.

Table 3: Impact of different buffer source on differential leukocytic counts, phagocytosis, lysozomal and bacteriocidal activity of growing barki lambs fed on rations containing different roughage : concentrate ratios.

Items / Treatments	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Neutrophil / Lymphocyte Ratio	Phagocytosis (%)	Lysozomal activity	Bacteriocidal activity
T1	64.33± 1.67[ab]	29.17± 1.76[b]	3.33± 0.88	2.17± 0.33	2.20± 0.16[b]	49.33± 2.96[cde]	0.680± 0.05	31.17± 4.58
T2	63.00± 2.31[ab]	33.00± 2.65[ab]	2.67± 0.67	1.33± 0.33	1.95± 0.24[ab]	59.67± 1.45[ab]	0.573± 0.13	42.50± 13.6
T3	69.67± 1.86[a]	27.33± 1.45[b]	2.33± 0.33	0.67± 0.33	2.57± 0.2[a]	59.33± 2.96[ab]	0.573± 0.12	42.30± 12.56
T4	63.67± 2.73[b]	34.67± 2.4[a]	2.00± 0.58	0.67± 0.67	1.84± 0.18[b]	54.33± 3.18[bc]	0.377± 0.13	62.47± 13.47
T5	62.33± 3.76[b]	34.00± 3.21[ab]	3.00± 1.00	0.67± 0.33	1.89± 0.3[b]	52.67± 3.28[bcd]	0.323± 0.02	67.37± 2.31
T6	65.00± 1.53[ab]	30.67± 2.33[ab]	2.33± 0.33	2.00± 0.58	2.15± 0.21[ab]	66.67± 2.03[a]	0.453± 0.15	54.57± 15.65
T7	62.67± 1.33[ab]	35.00± 1.15[b]	1.67± 0.33	0.67± 0.33	1.94± 0.12[ab]	42.67± 2.33[e]	0.477± 0.04	56.73± 2.49
T8	63.00± 0.58[ab]	32.33± 0.88[ab]	3.67± 0.88	1.00± 0.58	1.95± 0.06[ab]	45.33± 3.71[de]	0.510± 0.08	49.27± 8.22
T9	65.67± 2.03[ab]	30.00± 2.52[b]	2.33± 0.33	2.00± 0.58	2.23± 0.24[ab]	41.33± 2.33[e]	0.417± 0.16	57.67± 12.73
P value R:C ratio	0. 156	0.115	0. 816	0.238	0.165	0.000	0.070	0.051
Buffer	0.080	0.073	0. 263	0.275	0.045	0.022	0.893	0.946
Interaction	0. 413	0.477	0. 373	0.229	0.477	0. 019	0. 837	0.785

Values are expressed as Mean±SE. Means in the same column different superscript letter are significantly at ($P<0.05$). T1: R60:C40 (Without buffer). T2: R60:C40 (Sodium bicarb). T3: R60:C40 (Sodium bentonite). T4: R40: C60 (Without buffer). T5: R40: C60 (Sodium bicarb). T6 R40: C60 (Sodium bentonite). T7: R20: C80 (Without buffer). T8: R20: C80 (Sodium bicarb). T9 R80: C20 (Sodium bentonite).

Table 4: Impact of different buffer source on rumen (NH_3-N), volatile fatty acids (VFA) and rumen pH of growing barki lambs fed on rations containing different roughage : concentrate ratios.

Items / Treatments	Rumen (NH_3-N) (mg/dl)	Total VFA (mmol/dl)	Acetic %	Propionic %	Butyric %	Rumen PH			
						0H*	2H*	4H*	6H*
T1	9.77±0.55^{b}	76.6±0.98^{c}	59.07±1.53^{b}	20.77±0.73^{b}	5.80±0.49^{a}	6.13±0.07^{a}	6.53±0.13^{abc}	6.50±0.15^{a}	6.80±0.06^{a}
T2	8.40±0.5^{b}	79.6±0.6b^{c}	59.60±2.8^{ab}	22.65±0.35^{ab}	5.80±0.2^{a}	6.05±0.05^{a}	6.65±0.05^{ab}	6.45±0.05^{a}	6.90±0.10^{a}
T3	9.43±1.1^{b}	78.8±1.38^{bc}	61.47±1.28^{ab}	22.17±1.19^{ab}	5.73±0.18^{a}	6.10±0.06^{a}	6.73±0.03^{a}	6.53±0.03^{a}	6.77±0.03^{a}
T4	11.0±0.45^{ab}	81.1±1.94^{bc}	64.67±0.88^{ab}	23.77±0.23^{a}	5.41±0.36^{ab}	6.17±0.03^{a}	6.27±0.09^{cde}	6.17±0.03^{bc}	6.50±0.06^{b}
T5	10.47±0.39^{b}	82.1±2.16^{b}	65.60±2.55^{a}	22.17±0.8^{ab}	5.50±0.15^{a}	6.13±0.03^{a}	6.30±0.25^{bcd}	6.20±0.06^{b}	6.90±0.06^{a}
T6	9.63±0.48^{b}	81.9±1.89^{b}	66.80±1.01^{a}	23.53±0.79^{a}	5.07±0.18^{ab}	6.07±0.03^{a}	6.27±0.09^{cde}	6.07±0.03^{bc}	6.73±0.07^{a}
T7	13.40±1.18^{a}	87.5±0.81^{a}	64.67±2.4^{ab}	22.67±0.33^{ab}	5.33±0.35^{ab}	5.83±0.07^{b}	5.60±0.06^{f}	6.00±0.06^{bc}	6.27±0.03^{c}
T8	11.47±0.69^{ab}	90.1±0.43^{a}	63.00±2.31^{ab}	23.67±0.88^{a}	4.54±0.26^{b}	5.87±0.09^{b}	5.90±0.1^{ef}	5.97±0.03^{c}	6.20±0.06^{c}
T9	9.77±1.21^{b}	91.4±1.3^{a}	64.67±2.03^{ab}	23.67±0.88^{a}	4.53±0.29^{b}	5.73±0.09^{b}	5.93±0.15^{def}	6.00±0.06^{bc}	6.43±0.03^{b}
P value									
R:C ratio	0.011	0.000	.008	0.077	0.004	0.000	0.000	0.000	0.000
Buffer	0.054	0.121	.532	0.521	0.275	0.315	0.207	0.916	0.010
Interaction	0.308	0.834	.946	0.306	0.533	0.718	0.716	0.613	0.001

Values are expressed as Mean±SE. Means in the same column different superscript letter are significantly at ($P<0.05$). *H: Hours. T1: R60:C40 (Without buffer). T2: R60:C40 (Sodium bicarb). T3: R60:C40 (Sodium bentonite). T4: R40: C60 (Without buffer). T5: R40: C60 (Sodium bicarb). T6 R40: C60 (Sodium bentonite). T7: R20: C80 (Without buffer). T8: R20: C80 (Sodium bicarb). T9 R80: C20 (Sodium bentonite).

Intestinal morphology

The obtained data in **Table 5** and **Figures 1, 2 and 3** indicated that duodenal villi length and crypt depth significantly ($P<0.05$) increased with an increase of dietary concentrate level from 40 to 60%, while they increased ($P\geq0.05$) when dietary concentrate level increased from 40 to 80%. Both buffer supplementation to R60:C40 or R40:C60 ratios containing diets significantly increased villi length and crypt depth compared to those obtained by Barki lambs that are fed the same diet without buffer.

Table 5: Impact of different buffer source on intestinal histopathology of growing barki lambs fed on rations containing different roughage : concentrate ratios.

Items / Treatments	Villi length (µm)	Crypt depth (µm)	Villi crypt depth ratio
T1	898.89± 32.01[e]	263.31± 12.53[e]	3.42± 0.1[c]
T2	983.80± 42.32[e]	279.76± 8.25d[e]	3.51± 0.13[abc]
T3	1111.61± 41.27[cd]	300.72± 5.17[cd]	3.69± 0.08[ab]
T4	1159.45± 39.61[c]	324.84± 10.55[c]	3.57± 0.1[abc]
T5	1378.31± 24.91[b]	357.72± 8.88[b]	3.86± 0.13[a]
T6	1558.00± 34.08[a]	417.20± 6.32[a]	3.73± 0.1[ab]
T7	1005.74± 20.06[de]	285.92± 12.61[de]	3.53± 0.14[abc]
T8	939.07± 25.67[e]	293.06± 21.2[cde]	3.24± 0.19[cd]
T9	924.69± 56.37[e]	303.58± 6.98[cd]	3.04± 0.13[d]
P value			
R:C ratio	0.001	0.001	0.878
Buffer	0.001	0.001	0.001
Interaction	0.001	0.033	0.028

Values are expressed as Mean±SE. Means in the same column different superscript letter are significantly at (P<0.05). T1: R60:C40 (Without buffer). T2: R60:C40 (Sodium bicarb). T3: R60:C40 (Sodium bentonite). T4: R40: C60 (Without buffer). T5: R40: C60 (Sodium bicarb). T6 R40: C60 (Sodium bentonite). T7: R20: C80 (Without buffer). T8: R20: C80 (Sodium bicarb). T9 R80: C20 (Sodium bentonite).

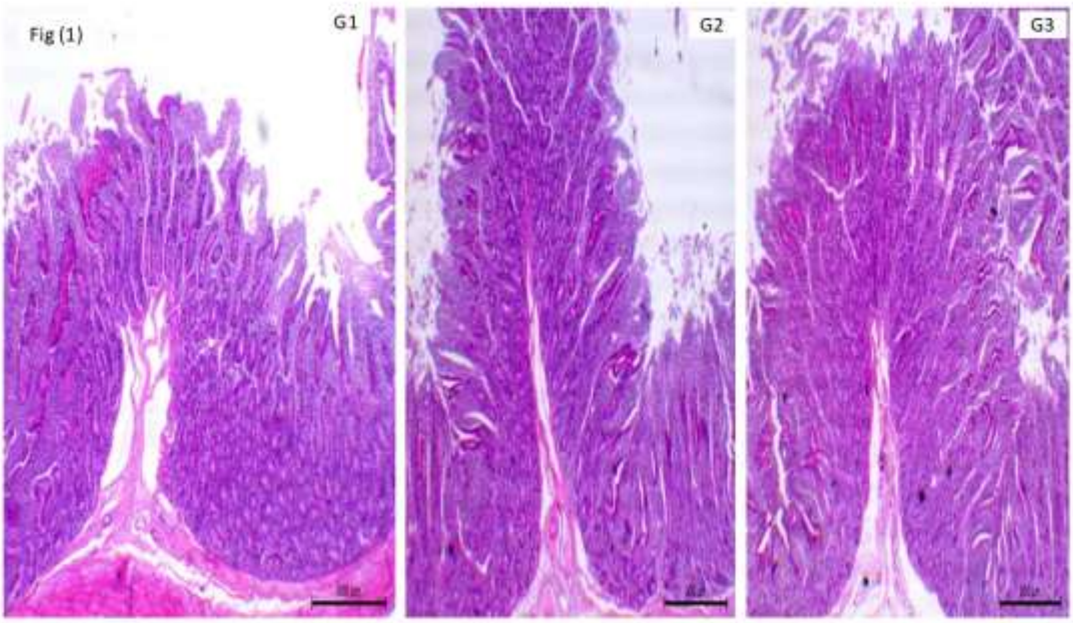

Fig.1: Photomicrograph of intestinal section of sheep of G1 (lambs were fed on R60:C40 containing diet without buffer) showing normal intestinal mucosa consisted of normal intestinal glands, (HandE stain), X40, bar= 500 µm. Photomicrograph of intestinal section of sheep of G2 (lambs were fed on R60:C40 containing diet with sodium bicarbonate buffer) showing normal intestinal mucosa consisted of normal intestinal folds consisted of normal intestinal glands, (HandE stain), X40, bar= 500 µm. Photomicrograph of intestinal section of sheep of G3 (lambs were fed on R60:C40 containing diet with sodium bentonite buffer) showing normal intestinal mucosa consisted of normal intestinal fols with increase the length of the mucosa, (HandE stain), X40, bar= 500 µm.

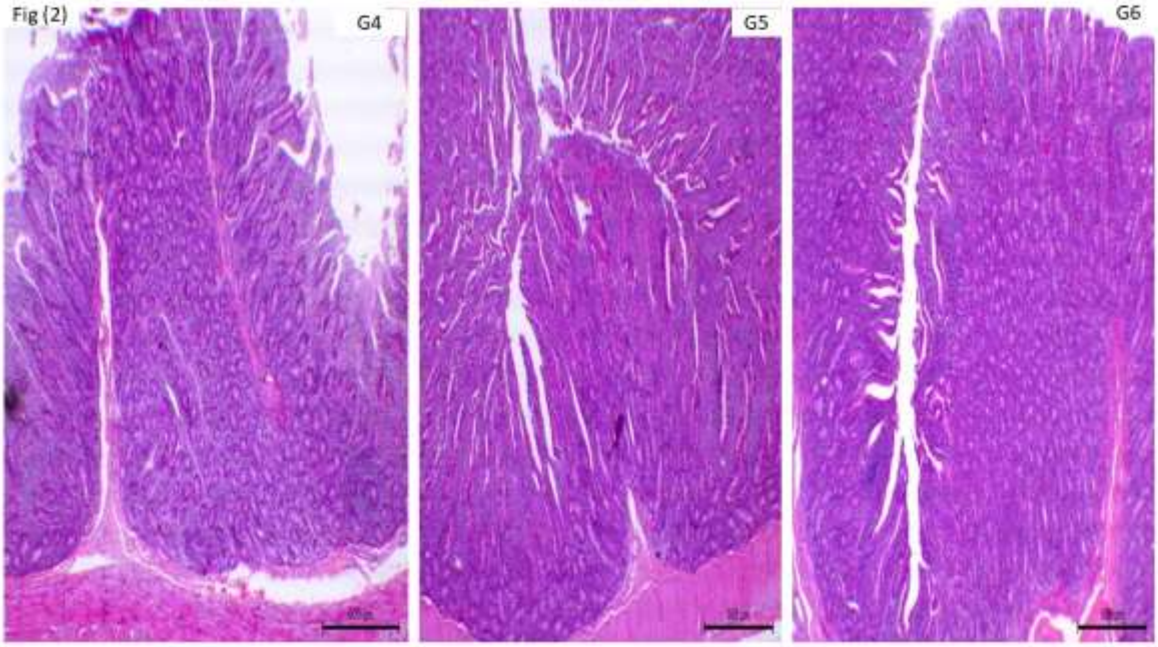

Fig. 2: Photomicrograph of intestinal section of sheep of G4 (lambs were fed on R40:C60 containing diet without buffer) showing normal intestinal mucosa consisted of normal intestinal gland with marked increase the length of the mucosa (HandE stain), X40, bar= 500 µm. Photomicrograph of intestinal section of sheep of G5 (lambs were fed on R40:C60 containing diet with sodium bicarbonate buffer) showing normal intestinal mucosa with marked increae of their length (HandE stain), X40, bar= 500 µm. Photomicrograph of intestinal section of sheep of G6 (lambs were fed on R40:C60 containing diet with sodium bentonite buffer) showing marked increase of intestinal mucosa length, (HandE stain), X40, bar= 500 µm.

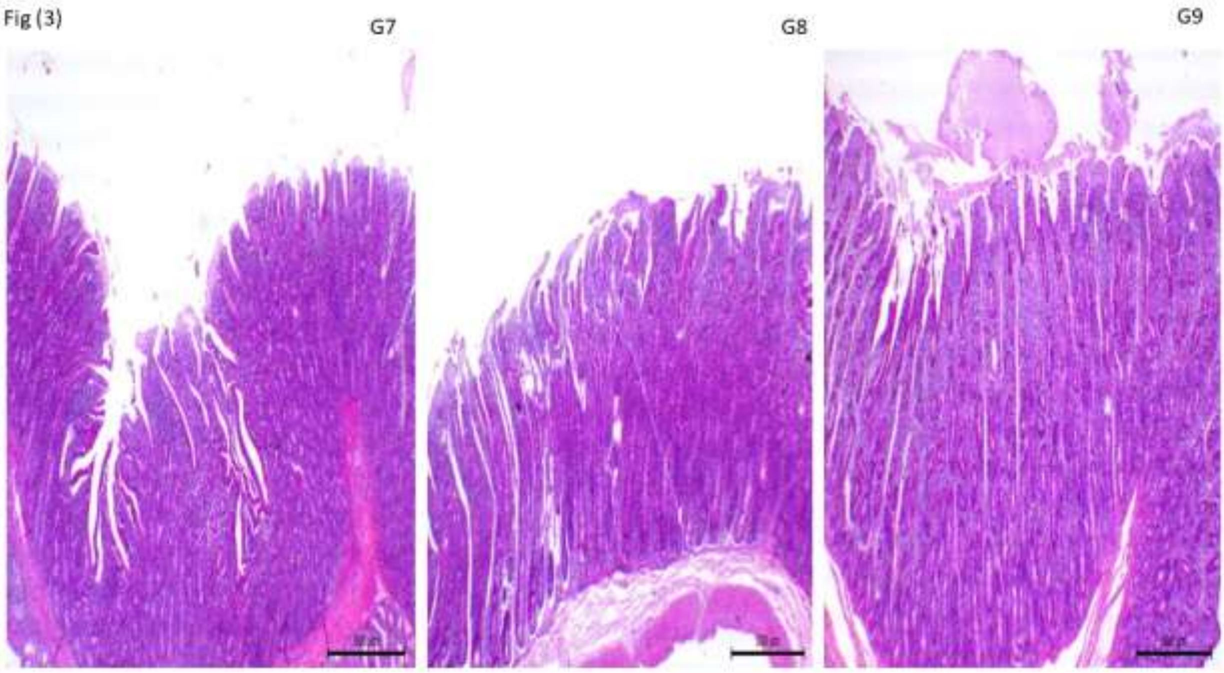

Fig.3: Photomicrograph of intestinal section of sheep of G7 (lambs were fed on R20:C80 containing diet without buffer) showing normal intestinal mucosa consisted of normal intestinal glands, (HandE stain), X40, bar= 500 µm. Photomicrograph of intestinal section of sheep of G8 (lambs were fed on R20:C80 containing diet with sodium bicarbonate buffer) showing normal intestinal mucosa consisted of normal intestinal glands, (HandE stain), X40, bar= 500 µm. Photomicrograph of intestinal section of sheep of G9 (lambs were fed on R20:C80 containing diet with sodium bentonite buffer) showing normal intestinal mucosa consisted of normal intestinal folds, (HandE stain), X40, bar= 500 µm.

DISCUSSION

The obtained results revealed that buffer supplementation with either sodium bicarbonate or sodium bentonite and/orhigh concentrate % enhanced growth performance. These findings are in linearity with **Aydin *et al.,* (2020)** who stated that 1% or 2% sodium bentonite supplementation increased total body gain and improved average feed conversion ratio, especially the lower supplementation level compared to control. Also, **Alhidary *et al.,* (2019**) showed that the different buffering agents' inclusion in the diets enhanced feed efficiency in growing Awassi lambs. In fattening lambs, dietary additions of 0.2% sodium bicarbonate and 0.05% magnesium oxide heightened the feed intake and daily weight gain (**Hashemi *et al.,* 2012**). Despite not directly affecting feed intake or digestion, common buffers can enhance food digestion in the rumen and small intestine (**Erdman, 1988**). Consistent with our results, **Azadbakht *et al.,* (2017**) concluded that adding 1.5% and 3% dietary bentonite improved live weight gain and FCR of Zandi lamb. Different buffers, either sesquisodium carbonate or sodium bicarbonate, had no significant impact on live weight, daily weight gain, dry matter intake and FCR of fattening lambs (**Asadi *et al.,* 2024**). **Oliveira *et al.,* (2016**) found that 3% bentonite addition to the ration didn't significantly affect the FCR of Merino lambs.

Our results are in agreement with **Oliveira *et al.,* (2020)** and **Jin and Zhou (2022),** who indicated that higher concentrate rations (30R:70C or 45R:55C) improved body weight gain and FCR compared to low concentrate (70R:30C or 65R:35C) fed groups. Similarly, **Dutta *et al.,* (2023)** reported that kids fed 2.1% of their live weight in a concentrate mixture exhibited higher body weight, followed by those fed 1.4% or 0.7%.

In the present study, R40:C60 with sodium bicarbonate recorded the best growth performance (the highest body weight and gain with the lowest FCR), followed by sodium bentonite with 40:60 and then 60:40 roughage concentrates ratios. In contrast to our findings, **Ameen *et al.,* (2023)** found that incorporating 2% sodium bicarbonate into the diet increased dry matter intake, digestibility, pH value, ruminal TVFA concentrations, and daily gain. Furthermore, **Tripathi *et al.,* (2004)** further indicated that adding sodium bicarbonate to a high-concentrate diet improved growth by boosting feed intake and lamb digestibility.

A slight decrease in neutrophil counts was noted as the roughage-to-concentrate (R:C) ratio declined from 60:40 to 40:60 and 20:80, likely due to diminished leukocyte production stemming from reduced energy and protein availability. This drop could also relate to thymus atrophy linked to malnutrition, as mentioned by **Fiske and Adams (1985).** Various immune-related measures, including antibody production, the neutrophil-to-lymphocyte (N/L) ratio, and the activity of peripheral blood mononuclear cells, are proposed as indicators of chronic stress in livestock **(Trevisi and Bertoni, 2009).** In this study, sodium bentonite supplementation led to a steady rise in the N/L ratio, suggesting heightened stress levels. An elevated N/L ratio may indicate an underlying health challenge and a compromised immune system, often observed in animals with poor health **(Hyun *et al.,* 2005).**

Rumen (NH_3-N) represents the end product of protein and non-protein nitrogen breakdown in feed and serves as the primary source of nitrogen for rumen microorganisms to synthesize microbial protein. In the current study, a high-concentrate diet significantly raised rumen (NH_3-N) levels, consistent with previous studies (**Yang *et al.,* 2001; Liu *et al.,* 2019**). The increased dietary concentrate likely enhanced rumen nitrogen availability so that it fostered higher ruminal (NH_3-N) levels **(Moorby *et al.,* 2006).**

Supplementing the total mixed ration (TMR) of growing lambs with buffers such as sodium bicarbonate or bentonite was found to lower ruminal (NH_3-N) concentrations compared to TMR without these buffers. This reduction was not statistically significant at the R:C ratio (60:40) but became significant as the R:C ratios decreased. These findings align with those of **Farghaly *et al.,* (2019**), who indicated a trend towards reduced ruminal ammonia concentrations in sheep with the addition of sodium bicarbonate to their diets. Additionally, **Saleh *et al.,* (1999)** noted a decrease in ruminal NH_3-N levels when varying levels of bentonite were included in ruminant diets. The lower rumen (NH_3-N) concentrations might result from bentonite's ability to adsorb NH_3-N from the ruminal fluid at elevated levels and release it when levels drop (**Bartos *et al.,* 1982**).

High-concentrate diets correlated with decreased acetate levels and increased propionate production, causing a significant decrease in the acetate-to-propionate ratio **(Andrade and Schmidely, 2006; Polyorach *et al.,* 2014).** This alteration in fermentation patterns indicates enhanced energy utilization efficiency, likely contributing to the improved growth performance and feed efficiency **(Seema *et al.,* 2025).** Sodium bicarbonate ($NaHCO_3$) has been shown to boost ruminal microbial activity, thus increasing TVFA concentrations in rams **(Santra *et al.,* 2003).** The same study also recorded significant increases in both ruminal pH and TVFAs with higher dietary sodium bicarbonate levels.

It is a well-recognized fact that TMR demonstrated a lower rumen pH, likely as a result of accelerated fermentation along with an increased yield of volatile fatty acids (VFAs) (**Blanco *et al.,* 2015; Alhidary *et al.,* 2017; Alhidary *et al.,* 2019**). The ruminal pH has been recognized as a potential constraint when assessing the likelihood of induced acidosis. A rumen pH range between 6.2 and 7.0 is usually regarded as optimal for sustaining a stable microbial ecosystem and facilitating normal rumen fermentation (**Wang, 2013**). In this investigation, lambs fed diets with R:C ratios of 60:40 and 40:60 had ruminal pH ranges from 6.13 to 6.80 and 6.03 to 6.67, respectively. These findings indicate that elevating the concentrate content to 60% in the TMR remained within the standard range for rumen fermentation and did not trigger ruminal acidosis, which is conventionally defined as a pH below 6.0 (**Liu *et al.,* 2019; Zhang *et al.,* 2023**).

Conversely, lambs consuming a high-concentrate diet (R20:C80) exhibited lower ruminal pH values, ranging from 5.73 to 5.83 during the initial two hours following feeding, suggesting the onset of subacute acidosis. The ruminal pH values observed in this study differ from those documented in earlier research (**Chen *et al.,* 2015**), likely due to a multitude of influencing factors such as the dietary concentrations (**Lettat et al., 2010; Minuti *et al.,* 2014**), the timing of rumen fluid collection in relation to feeding (**Lettat *et al.,* 2010**), and the catheter insertion depth during sampling (**Li *et al.,* 2009**). Overall, the noted decline in ruminal pH with rising concentrate levels may be associated with the higher starch content within the ration.

Both sodium bicarbonate and sodium bentonite elevate ruminal fluid pH, thereby mitigating the adverse effects of high concentrate on rumen acidity. These findings correspond with those reported by **Johnson *et al.,* (1988)**, who noted that the incorporation of bentonite into sheep diets significantly ($P<0.05$) elevated rumen pH. Conversely, **Grabherr *et al.,* (2009)** indicated no notable alteration in ruminal pH levels when zeolite was introduced at 10 or 20 g/kg DM into the diets of cattle.

Furthermore, the present findings imply that sodium bicarbonate typically outperforms sodium bentonite in stabilizing ruminal pH, especially in lambs consuming diets with R40:C60 or R60:R40. However, in lambs receiving a higher concentrate diet (R20:C80), sodium bentonite was more effective at sustaining ruminal pH stability. Similarly, **Cruywagen *et al.,* (2004)** and **Cruywagen *et al.,* (2015)** found that AcidBuf and sodium bicarbonate increased rumen pH in dairy cows, with AcidBuf being more successful than sodium bicarbonate at stabilizing ruminal pH. Sodium bentonite, which encompasses various minerals, plays a role in enhancing ruminal pH, particularly under high-concentrate feeding situations, thereby boosting feed efficiency (**Alhidary *et al.,* 2019**).

Villus height and crypt depth are widely known as essential morphological indicators of small intestine well-being, particularly in the duodenum of animals (**Sun *et al.,* 2018**). These factors reflect the intestine's ability for nutrient digestion and absorption (**McCoard *et al.,* 2020**). An uptick in villus height correlates with improved nutrient absorption (**Short and Derrickson, 2020**), while a greater crypt depth is positively linked to a higher rate of cellular regeneration (**Bogusławska-Tryk *et al.,* 2020**). However, the current results deviate from those reported by **Zhou *et al.,* (2022),** who noted a decline ($P \geq 0.05$) in duodenal villus length with increasing dietary concentrate levels in black Tibetan sheep. The reduction in villus height and crypt depth at elevated concentrate levels (R20:C80) may be associated with insufficient dietary structural carbohydrates (fiber). Diets rich in roughage, recognized for their excellent absorption and retention capabilities of water, improve satiety and stimulate intestinal peristalsis, thereby fostering intestinal development (**Loor *et al.,* 2004**).

CONCLUSION

Feeding lambs with a high-concentrate (80%) diet without buffers resulted in the lowest growth performance; meanwhile, the best body weight gain and feed conversion ratio were obtained when we fed sodium bicarbonate buffer with a medium concentrate level (60%). Feeding buffers with or without a higher concentrate ratio enhanced villi length and crypt depth, thereby improving the absorptive capacity and bolstering rumen health through reduced rumen ammonia levels, overcoming the hazards associated with concentrate on rumen pH and total feed intake, ultimately enhancing lamb growth performance alongside improved immunity. Sodium bicarbonate with high concentrate ratios (60 and 80%) outperformed the sodium bentonite; the reverse was true for lower concentrate ratios (40%). For enhancement of lambs' growth performance (increase body weight gain and reduce FCR), immunity (lower bacteriocidal and heightened lysosomal activities), and rumen health (decrease pH and ammonia, thus improving microflora activities and volatile fatty acid production), we recommended feeding a 40R:60C ratio with a sodium bicarbonate buffer.

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Conflicts of interest

The authors declare that they have no competing interests.

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