



# Rumen Discoloration of Growing Lambs Fed with Diets Containing Different Levels of Neutral Detergent Fibre

Ibrahim A. Alhidary<sup>1,\*</sup>, Mutassim M. Abdelrahman<sup>1</sup>, Riyadh S. Aljumaah<sup>1</sup>, Abdullah H. Alyemni<sup>2</sup>, Moez A. Ayadi<sup>1,3</sup> and Mohamed Y. Al-Saiady<sup>2</sup>

<sup>1</sup>Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

<sup>2</sup>Arabian Agricultural Services Company, P.O. Box 53845, Riyadh 11593, Saudi Arabia

<sup>3</sup>Département de Biotechnologie Animale, Institut Supérieur de Biotechnologie de Beja, Université de Jendouba, B.P. 382, Av. Habib Bourguiba, 9000 Beja, Tunisia

## ABSTRACT

A study was conducted to investigate the effects of different total mixed ration diets (TMRs) with three different levels of NDF on growth performance, metabolic profile and rumen characteristics in Naemi lambs. Fifty growing Naemi lambs at age of three months with an average bodyweight of  $27.77 \pm 1.09$  kg were randomly distributed to five different treatments, ten lambs each, as follow: 1. Alfalfa hay and barley grain (ABG); 2. Pelleted alfalfa hay and barley (PAB); 3. TMR1 (25.67% NDF); 4. TMR2 (30.88% NDF); and 5. TMR3 (55.93% NDF). Lambs were housed in an individual pen for 90 days. Lambs were weighed every two weeks and feed intake was recorded weekly. Rumen fluid samples were collected monthly after 2, 4 and 8 hours of feeding for pH, VFAs and  $\text{NH}_3\text{-N}$  determination. Blood samples were collected monthly for different metabolic analysis. At the end of the trial, six lambs from each group were randomly selected and slaughtered for rumen coloring. The results of this trial showed a significantly higher total body weight gain in lambs fed TMR1 and TMR 2. A significantly higher FCR in lambs fed TMR3 compared with all other treatments. The rumen color of lambs fed TMR2 was significantly ( $P < 0.01$ ) lighter compared with all other groups except lambs fed ABG. Pelleting the alfalfa hay and barley affect the rumen color to be darker compared to ABG. Regarding the rumen fluid pH, lambs in ABG and TMR2 groups were significantly higher after eight hours of feeding compared with all other dietary groups. In conclusion, feeding growing lambs with TMRs containing 30% NDF, leads to obtain a safer and suitable rumen characteristics, microbial fermentation and growth performance.

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## Authors' Contribution

IAA and MMA designed and supervised the study and wrote the article. RSA did statistical analysis, MAA was incharge and responsible of lab work. AHA and MYA conducted the research and edited the paper.

## Key words

Growing lambs, Metabolic profile, Performance, Rumen characteristics, Total mixed rations.

## INTRODUCTION

The well balance diets for ruminants must be considered carefully to cover the nutrient requirements of the animal to optimize their productivity (Alhidary *et al.*, 2016a; Abdelrahman *et al.*, 2017a). In the last few years, to increase animals' productivity, the traditional extensive and semi intensive of livestock production systems have been converted to intensive system in Saudi Arabia (Alhidary *et al.*, 2016b). Therefore, feeding total mixed ration (TMR) diets could be a perfect solution to fill the shortage gap of animals' feed requirements (Abdelrahman *et al.*, 2017b). In formulating a TMR, focus is mainly concentrated on covering the energy, protein, minerals and vitamins requirements (Alhidary *et al.*, 2016c). Cellulose, hemicellulose and lignin (NDF) are also the major components of the feed

that affect the feed volume and saliva production (Varga *et al.*, 1998). National Research Council (2001) recommends that 25% of dietary dry matter has to be NDF with at least 75% from a forage portion. Kozloski *et al.* (2006) found that feeding 30% NDF is very suitable to enhance the performance and productivity of feedlot lambs. Special consideration regarding the required NDF must be followed when formulating the TMRs rations for ruminant animals. Moreover, feeding TMR with high fermentable carbohydrate may increase the risk of subacute or acute ruminal acidosis by increasing the propionic and lactic acid, thus decreasing the ruminal pH and consequently affect ruminant animal health and productivity (Bodas *et al.*, 2007; Enemark, 2008). Moreover, the physical factors (pellets), NDF sources and particle size of the ration as well as the chemical factors can cause a dark-brownish color of rumen epithelium tissues (Alhidary *et al.*, 2016b). The risk of ruminal acidosis can be reduced by adopting feeding regime through increasing or decreasing the dietary intake of daily NDF by growing lambs. So,

\* Corresponding author: ialhidary@ksu.edu.sa  
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defining the NDF requirements by growing animal is very crucial to control the rumen pH and consequently reduce the effect of feeding TMR on the ruminal fermentation.

This experiment was conducted to study the effect of feeding TMR with different NDF levels, compared to the traditional feeding system on the general performance of growing lambs and rumen characteristics.

## MATERIALS AND METHODS

A total of fifty, three months old growing Naemi male lambs (mean initial BW,  $27.77 \pm 1.09$  kg) were randomly chosen in this experiment. On arrival, lambs were immediately weighed, ear tagged, vaccinated against clostridial diseases and treated for internal and external parasites. Thereafter, the animals were individually housed in shaded pens. Each pen was equipped with a feed trough and a 10-L plastic water bucket. On day 1 of the experiment, lambs were randomly divided, into 5 dietary treatments, (ten lambs per treatment). The dietary treatments were: 1) control group: lambs fed a traditional feeding diet (alfalfa hay and barley at 1:1; ABG); 2) Pelleted alfalfa hay and barley group (PAB); 3) TMR diet containing 25.67% of NDF (TMR1); 4) TMR diet containing 30.88% of NDF (TMR2) and 5) TMR diet containing 55.99% of NDF (TMR3). The TMRs were isocaloric ( $GE = 4333.0 \pm 39.13$  Kcal/kg DM basis) and isonitrogenous ( $CP = 13.70 \pm 0.44\%$  DM basis; AOAC, 1990). The basal feed composition is

given in Table I.

Lambs were offered one of the five diets and water as *ad libitum*. Diets were sampled before the experiment commenced and weekly during the experiment, and thereafter, the samples were frozen at  $-20^\circ\text{C}$ . At the end of the experiment, the samples were pooled and analyzed for nutrient composition according to methods described by van Soest *et al.* (1991) and AOAC (1990). The offered feed and refusals were measured weekly and then feed intake was calculated, on a DM basis, for each lamb. Animals were weighed before feeding at 0700 hour on day 1 of the study and every two weeks thereafter until the end of each study. Feed conversion ratio (FCR) for each lamb was calculated and expressed as the amount of DM required per kg of bodyweight gain.

Blood samples were collected via the jugular vein from all lambs on days 1, 30, 60 and 90. At each collection, 10 mL aliquots of blood were taken into plain vacutainer tubes for serum collection. Serum was obtained by centrifugation at  $2,400 \times g$  for 15 min at  $4^\circ\text{C}$  and will then be frozen at  $-20^\circ\text{C}$  until further analysis. Serum concentrations of glucose, non-esterified fatty acid (NEFA), total protein, albumin, urea, total cholesterol and triglyceride were analyzed using commercial kits (Randox Laboratories, Antrim, United Kingdom) and a microplate reader (Multiskan EX, Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer's procedures.

**Table I.- Basal feed composition.**

Ingredients, % of dietary DM	ABG	PAB	TMR1	TMR2	TMR3
Barley grain	50	50	18.00	18.00	18.00
Alfalfa	50	50	3.00	4.50	5.50
Wheat	-	-	18.00	17.00	18.4
Palm Kernel Meal	-	-	29.92	29.00	28.50
Soya Hulls	-	-	20.00	19.00	18.0
Wheat Bran	-	-	12.03	13.5	13.0
Salt	-	-	0.47	0.47	0.47
Limestone	-	-	2.58	2.58	2.58
Molasses	-	-	7.85	7.85	7.85
Commercial Premix <sup>1</sup>	-	-	0.15	0.15	0.15
<b>Chemical analysis (%)</b>					
Dry matter	18.1 $\pm$ 1.2	17.95 $\pm$ 0.9	90.04 $\pm$ 0.03	88.34	91.21
Crude Protein	17.95 $\pm$ 0.5	18.2 $\pm$ 0.1	13.5 $\pm$ 0.01	14.00	13.98
Crude Fibre	27.00 $\pm$ 0.4	26.75 $\pm$ 0.2	12.00 $\pm$ 0.01	11.65	12.03
Ether extract	1.8 $\pm$ 0.01	1.84 $\pm$ 0.01	2.45 $\pm$ 0.01	2.54	2.5
Neutral detergent fibre	42.12 $\pm$ 0.7	42.8 $\pm$ 0.3	25.67 $\pm$ 0.15	30.88	55.93
Acid detergent fibre	20.98 $\pm$ 0.1	20.4 $\pm$ 0.4	25.76 $\pm$ 0.2	25.11	25.42
Ash	11.23 $\pm$ 0.01	11.12 $\pm$ 0.02	8.96 $\pm$ 0.02	8.69	8.54

ABG, alfalfa hay and barley grain; PAB, pelleted alfalfa hay and barley; TMR1, total mixed ration diet containing 25.67% of NDF; TMR2, total mixed ration diet containing 30.88% of NDF; TMR3, total mixed ration diet containing 55.93% of NDF for 90 days.

Approximately 100 ml of rumen fluid sample from different 3 sets (front, middle, and back) was collected from 6 lambs per treatment, using an oral stomach tube, before the morning feeding (0 hour) and then 2, 4 and 8 h post feeding on day 1, and monthly thereafter until the end of the experiment, and also after slaughter. Rumen fluid samples were analyzed immediately using a microprocessor pH-meter (Model pH 211, Hanna Instruments, Woonsocket, RI, USA). Rumen fluid concentrations of total volatile acid, acetate, propionate, butyrate, valeric and isovaleric were analyzed using an Agilent series gas chromatograph with a special capillary column (Agilent Technologies Inc., Wilmington, DE) based on methodology from Supelco Inc. (1975). The rumen fluid ammonia ( $\text{NH}_3\text{-N}$ ) concentrations was measured using spectrophotometer (Perkin Elmer, Waltham, MA, USA) after sample prepared by ammonia reaction with alkaline hypochlorite and phenol in the presence of a catalyst (sodium nitroprusside) to form indophenol (blue; Berthelot reaction).

At the end of the experiment (day 90), 6 lambs from each treatment were firstly deprived of feed and water for 16 h and then slaughtered. Rumen tissues were removed and process by the method as described by Alhidary *et al.* (2016c). The color of rumen was monitored with the help of Minolta Chroma Meter (Konica Minolta, CR-400- Japan) with a CIELAB Color System for the color values ( $L^*$  = value designates lightness;  $a^*$  and  $b^*$  = color coordinates).

#### Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of Statistical Analysis System Institute, Inc. (SAS, 2002). Means of each treatment were compared by using protected least significant differences (LSD) and significance was determined at  $P < 0.05$ ; the values are presented in the tables with pooled standard error of the means.

## RESULTS

#### Growth rate, DMI and FCR

The effects of feeding different commercial TMR diets containing different levels of dietary NDF compared with the traditional diets (alfalfa hay plus barley grain) on the growth performance of growing lambs are presented in Table II. There was a significant effect ( $P < 0.05$ ) of dietary treatments on growth rate, including final BW, BW change and ADG. Lambs on the PAB and TMR1 diets had greater ( $P < 0.05$ ) growth indicators and more significant improvement ( $P < 0.01$ ) in FCR when compared with other groups. While, feeding lambs on the TMR diet containing NDF at level of 30.88% resulted ( $P < 0.02$ ) in a decreased

in DMI when compared with lambs in the other treatments.

**Table II.- Means (kg) body weight (BW), average daily gain (ADG), dry matter intake (DMI) and feed conversion ratio (FCR) of growing lambs fed diets containing different levels of NDF.**

Item (kg)	Treatment					SEM <i>P</i>	
	ABG	PAB	TMR1	TMR2	TMR3	-val.	
Initial BW	27.22	29.80	27.78	27.30	26.67	0.75	0.49
Final BW	44.84 <sup>b</sup>	47.64 <sup>a</sup>	46.26 <sup>a</sup>	45.97 <sup>b</sup>	44.49 <sup>b</sup>	1.55	0.03
BW change	17.62 <sup>b</sup>	17.84 <sup>b</sup>	18.48 <sup>a</sup>	18.67 <sup>a</sup>	17.73 <sup>b</sup>	0.84	0.04
ADG	0.196 <sup>b</sup>	0.198 <sup>b</sup>	0.205 <sup>a</sup>	0.207 <sup>a</sup>	0.197 <sup>b</sup>	0.06	0.03
DMI	1.33 <sup>a</sup>	1.32 <sup>a</sup>	1.31 <sup>a</sup>	1.27 <sup>b</sup>	1.34 <sup>a</sup>	0.04	0.02
FCR	6.79 <sup>a</sup>	6.67 <sup>a</sup>	6.39 <sup>b</sup>	6.14 <sup>b</sup>	6.80 <sup>a</sup>	0.21	0.01

<sup>a,b</sup> within a row, means without a common superscript differ ( $P < 0.05$ ). <sup>1</sup> Values are for lambs ( $n = 50$ ) fed ABG, alfalfa hay and barley grain; PAB, pelleted alfalfa hay and barley; TMR1, total mixed ration diet containing 25.67% of NDF; TMR2, total mixed ration diet containing 30.88% of NDF; TMR3, total mixed ration diet containing 55.93% of NDF for 90 days; SEM, standard error of means.

**Table III.- Effects of feeding different levels of NDF on color values of rumen and reticulum tissues of growing lambs.**

Color components <sup>2</sup>	Treatment					SEM <i>P</i>	
	ABG	PAB	TMR1	TMR2	TMR3	-val.	
<b>Rumen</b>							
$L^*$	46.61 <sup>a</sup>	43.15 <sup>b</sup>	38.46 <sup>c</sup>	45.19 <sup>a</sup>	39.59 <sup>c</sup>	0.73	0.01
$A^*$	6.05	3.36	3.74	6.15	3.57	0.34	0.21
$B^*$	18.58 <sup>a</sup>	12.54 <sup>b</sup>	9.21 <sup>c</sup>	14.59 <sup>b</sup>	11.08 <sup>b</sup>	0.75	0.01

<sup>a-c</sup> within a row, means without a common superscript differ ( $P < 0.05$ ). SEM, standard error of means. <sup>1</sup> Values are for lambs ( $n = 30$ ) fed ABG, alfalfa hay and barley grain; PAB, pelleted alfalfa hay and barley; TMR1, total mixed ration diet containing 25.67% of NDF; TMR2, total mixed ration diet containing 30.88% of NDF; TMR3, total mixed ration diet containing 55.93% of NDF for 90 days; <sup>2</sup> $L^*$ , Lightness;  $a^*$ , Redness;  $b^*$ , Yellowness.

Differences between treatments in the color values of rumen of growing lambs fed diet containing different levels of NDF presented in Table III and Figure 1. With the exception of ruminal color, value of lambs on the ABG and TMR2 had ( $P < 0.01$ ) greater value of lightness (lighter) than those of lambs on other treatments (PAB, TMR1 and TMR3 diets). The TMR1 diet decreased ( $P < 0.01$ ) the value of yellowness of rumen tissue compared with those of lambs in other treatments.

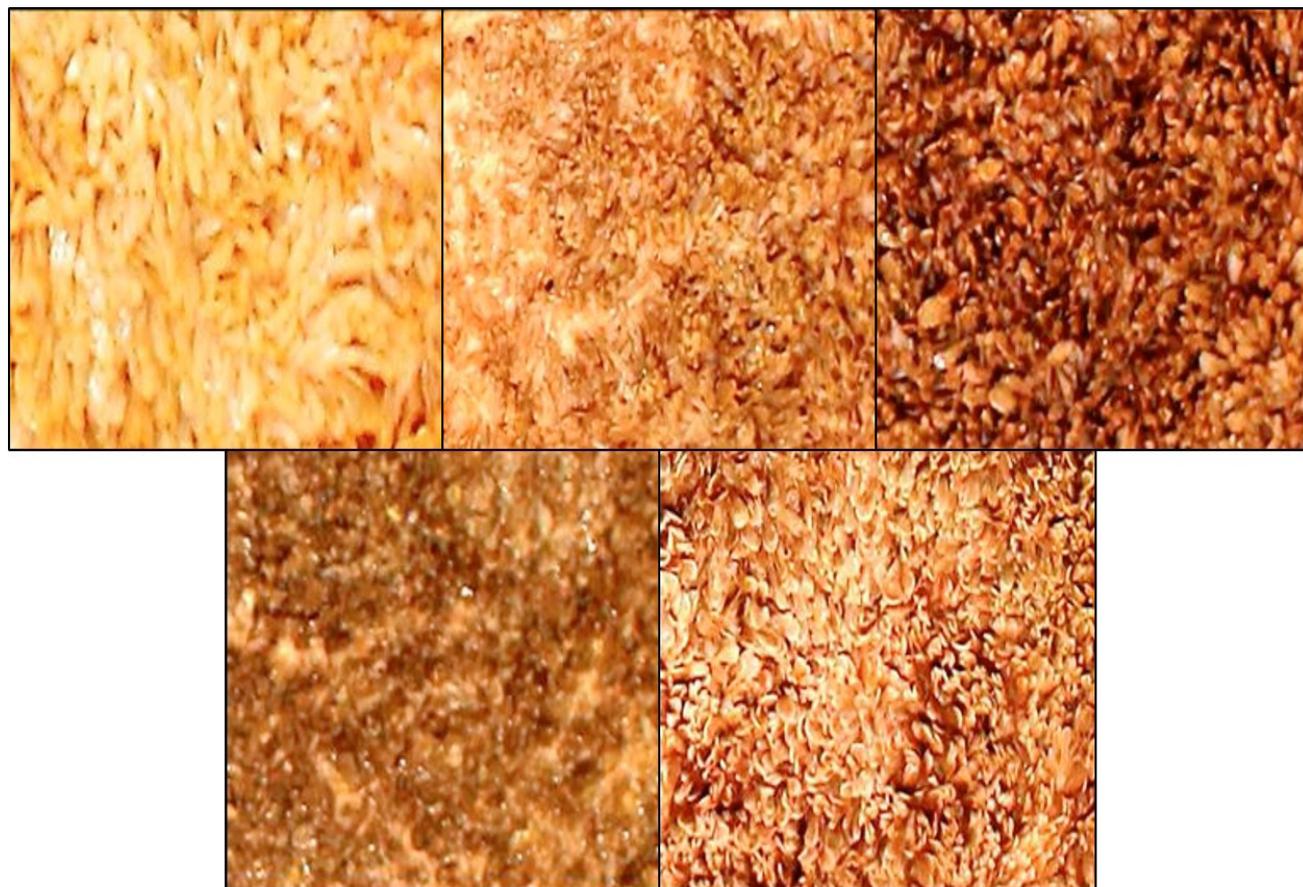


Fig. 1. Gross view of rumen of lambs fed. ABG, alfalfa hay and barley grain; PAB, pelleted alfalfa hay and barley; TMR1, total mixed ration diet containing 25.67% of NDF; TMR2, total mixed ration diet containing 30.88% of NDF; TMR3, total mixed ration diet containing 55.93% of NDF for 90 days.

**Table IV.- Effects of feeding different levels of NDF on pH values of ruminal fluid of growing lambs.**

Time after feeding, (h)	Treatment					SEM	P-value
	ABG	PAB	TMR1	TMR2	TMR3		
00:00	5.96 <sup>ab</sup>	6.62 <sup>a</sup>	5.83 <sup>b</sup>	5.81 <sup>b</sup>	5.77 <sup>b</sup>	0.10	0.02
02:00	6.21	5.91	6.02	6.50	5.98	0.11	0.56
04:00	6.70 <sup>a</sup>	6.00 <sup>b</sup>	5.85 <sup>b</sup>	6.37 <sup>ab</sup>	5.93 <sup>b</sup>	0.09	0.01
08:00	6.63 <sup>a</sup>	6.06 <sup>b</sup>	5.85 <sup>b</sup>	6.65 <sup>a</sup>	5.71 <sup>b</sup>	0.09	0.004
At slaughtering	5.64	5.40	5.57	5.87	5.57	0.17	0.81

<sup>ab</sup> within a row, means without a common superscript differ ( $P < 0.05$ ). <sup>1</sup> Values are for lambs ( $n = 30$ ) fed ABG, alfalfa hay and barley grain; PAB, pelleted alfalfa hay and barley; TMR1, total mixed ration diet containing 25.67% of NDF; TMR2, total mixed ration diet containing 30.88% of NDF; TMR3, total mixed ration diet containing 55.93% of NDF for 90 days. Rumen fluid samples were collected on days 1, 30, 60 and 90. SEM, standard error of means.

The pH values of ruminal fluid either after 2 h of feeding or at slaughtering time were not affected ( $P > 0.05$ ) by treatments. Lambs fed the TMR1 and TMR3 diets had less ( $P < 0.05$ ) pH values after 0, 4 and 8 hours of the feeding than those lambs on the other diets (Table IV).

Differences between treatments in the ruminal and serum concentrations of rumen fermentation and biochemical variables are presented in Tables V and VI, respectively. No differences were found ( $P > 0.05$ ) in the concentrations of rumen fermentation variable, and the serum concentrations of total protein and albumin. However, feeding lambs on the TMR2 diet resulted in increases in the serum triglyceride and urea concentrations and decreases in serum concentration of glucose. Lambs fed the TMR1 and TMR2 diets had the greatest ( $P < 0.01$ ) serum NDFA concentration. Feeding lambs on the TMR1 diet reduced ( $P < 0.002$  and  $0.03$ ) the serum concentrations of total cholesterol (Table V).

**Table V.- Mean ruminal concentrations of volatile fatty acid (g fatty acid / 100 g total VFAs) and ammonia-N (mg/dl) of lambs fed diets containing different levels of NDF.**

Analyte	Treatment					SEM	P-value
	ABG	PAB	TMR1	TMR2	TMR3		
Acetic	37.75	35.88	35.59	37.86	37.86	0.78	0.56
Propionic	33.82	35.45	41.21	30.98	34.67	4.11	0.14
Butyric	22.75	23.26	15.86	25.52	20.14	3.16	0.19
Valeric	2.48	3.05	3.25	3.26	4.05	0.25	0.22
Isovaleric	2.84	2.36	3.82	2.38	3.47	0.57	0.34
ammonia-N	23.39	25.00	24.18	25.41	18.18	3.75	0.12

<sup>a,b</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).  
<sup>1</sup>Values are for lambs ( $n = 30$ ) fed ABG, alfalfa hay and barley grain; PAB, pelleted alfalfa hay and barley; TMR1, total mixed ration diet containing 25.67% of NDF; TMR2, total mixed ration diet containing 30.88% of NDF; TMR3, total mixed ration diet containing 55.93% of NDF for 90 days. Rumen fluid samples were collected on days 1, 30, 60 and 90. SEM, standard error of means.

**Table VI.- Mean serum concentrations of metabolic profile in lambs fed diets containing different levels of NDF.**

Analyte	Treatment					SEM	P-value
	ABG	PAB	TMR1	TMR2	TMR3		
Glucose, mM	3.62 <sup>a</sup>	3.03 <sup>b</sup>	2.72 <sup>bc</sup>	2.51 <sup>c</sup>	3.02 <sup>b</sup>	0.16	0.001
NEFA, mM	0.38 <sup>b</sup>	0.44 <sup>b</sup>	0.69 <sup>a</sup>	0.61 <sup>a</sup>	0.47 <sup>b</sup>	0.05	0.01
Total Protein, g/L	63.45	57.43	53.99	52.89	53.99	3.28	0.31
Albumin, g/L	34.55	33.42	34.31	37.03	36.54	1.74	0.36
Urea, mM	5.84 <sup>bc</sup>	6.94 <sup>b</sup>	6.75 <sup>b</sup>	7.97 <sup>a</sup>	5.75 <sup>c</sup>	0.40	0.001
Total cholesterol, mM	2.17 <sup>a</sup>	1.83 <sup>ab</sup>	1.65 <sup>b</sup>	1.88 <sup>ab</sup>	1.73 <sup>ab</sup>	0.12	0.02
Triglyceride, mM	0.49 <sup>b</sup>	0.48 <sup>b</sup>	0.42 <sup>b</sup>	0.68 <sup>a</sup>	0.47 <sup>b</sup>	0.03	0.04
Ca, mM	2.22 <sup>a</sup>	2.13 <sup>a</sup>	2.16 <sup>a</sup>	1.76 <sup>b</sup>	2.08 <sup>a</sup>	0.06	0.001
P, mM	2.76 <sup>a</sup>	2.76 <sup>a</sup>	2.58 <sup>b</sup>	2.84 <sup>a</sup>	2.88 <sup>a</sup>	0.13	0.03

<sup>a-c</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).  
<sup>1</sup>Values are for lambs ( $n = 50$ ) fed ABG, alfalfa hay and barley grain; PAB, pelleted alfalfa hay and barley; TMR1, total mixed ration diet containing 25.67% of NDF; TMR2, total mixed ration diet containing 30.88% of NDF; TMR3, total mixed ration diet containing 55.93% of NDF for 90 days. Rumen fluid samples were collected on days 1, 30, 60 and 90. SEM, standard error of means.

## DISCUSSION

Very little information are known and understood regarding the proper NDF dietary requirement for growing lambs to maintain a suitable rumen healthy environment for microbial fermentation to cover their energy requirements for growth without negative effect on feed intake. By identifying the proper dietary NDF and digestibility values, TMR balancing can be more precise with higher nutrient digestibility and more predictable growth rate and general performance. In the present study, feeding TMRs with 25.67 and 30.88% NDF improved FCR in lambs. Previously, it was reported that manipulation of TMR with certain types of roughages and consequently NDF levels may improve feed intake and weight gain in fattening animals (Tufarelli *et al.*, 2012; Amaral *et al.*, 2015; Alhidary *et al.*, 2016). From the result of the present study, it could be inferred that feeding high NDF levels (55.93%) in the TMR caused a negative impact on FCR compared to other dietary groups which agreed with the findings reported by Blanco *et al.* (2014). These results could have negative implications on the ruminal conditions in the current study. In line with the previous reports, roughages consumption, to specific levels, support rumen epithelium and papillae, motility, increase the size and muscular development and promotes rumination (van Ackeren *et al.*, 2009; Alvarez-Rodriguez *et al.*, 2012). Some authors have supported the idea of higher NDF up to 30% in the roughages for enhanced rumen efficiency as observed in the current study (Beauchemin, 1991; Mertens, 1997). Therefore, it is inferred that feeding TMR with an acceptable NDF levels (about 30%) enhanced the feed intake and consequently weight gain in lambs. Therefore, an adequate amount of roughages are necessary in TMRs for proper structural and functional development of rumen (van Ackeren *et al.*, 2009). Furthermore, feeding NDF (30.88%; TMR2) improves the color of the rumen to be lighter compared with the lower NDF (25.67%; TMR1) and high NDF (55.93%; TMR3). This may be related mainly to the improvement in rumen pH which were significantly higher for lambs from the traditional feeding and TMR2 (30.88% NDF) groups (6.63 and 6.65, respectively) and a numerically higher profile of the volatile fatty acids especially acetic and butyric acids. Hamada *et al.* (1970) reported that dark coloration of the rumen epithelium tissues associated with the feeding of concentrate rations with high starch content, particularly pelleted rations compared with regular feeding. This support our finding that feeding TMR with lower NDF affect the pH and rumen tissues were darker compared with the regular traditional feeding and TMR with 30.88% NDF. Moreover, pelleting of the diets caused a darker color which reported in our study lambs rumen

fed pelleted diet (pelleted traditional feeding) compared with the regular traditional feeding.

The rumen pH is one of the most important determinants of ruminant performance since it impacts the fermentation pattern in the rumen. The inclusion of high fermented carbohydrate increases the risk of acidosis resulting in reducing feed intake and body weight gain (Krause and Combs, 2003). A high dietary NDF in the roughages, activate the chewing, salivation and rumination rate which leads to an increased rumen pH (Beauchemin, 1991; Mertens, 1997). Rumen pH at 5.7 has a negative effect on dry matter intake, NDF and protein digestibility and consequently lowering the total and individual volatile fatty acids (Calsamiglia *et al.*, 2002). The results of this study in term of the rumen pH supporting the above findings of increasing the NDF intake up to 30.88% feeding TMR after 8 hours of feeding, same as a traditional feeding. Furthermore, a significantly ( $P < 0.05$ ) higher total body gain and total dry matter intake in lambs fed TMR1 (25.67% NDF) and TMR2 (30.88% NDF) compared with other dietary groups. This finding completely agreed with the previous reported studies which is positively correlated with feeding NDF, to specific levels, with rumen pH through improving fermentation and production of microbial protein (Allen, 1997). From this study, it is well documented that NDF dietary levels and requirements play an important role on growing lambs' performance and rumen characteristics. Up to 30.88% NDF shows a significant improvement on growing lambs performance, but high levels such as 55.93% NDF negatively affect the performance and rumen characteristics.

As a general trend, high rumen pH as a result of feeding high NDF, to specific level, slows down the rumen fermentation pattern and lowers the volatile fatty acids production with high proportion of acetate to valerate and high acetate to propionate ratio in the rumen fluids (Carro *et al.*, 2000). Feeding concentrate base diets to ruminants leads to speed up the ruminal fermentation rate, high volatile fatty acids especially propionic acid and reduced rumen pH (Bodas *et al.*, 2007). This findings of this trial confirmed that high pH levels of rumen fluid of lambs fed high NDF (TMR3) compared to the recommended NDF (TMR1) and very high NDF (TMR 3). This means a very high NDF intake by growing lambs negatively affect the rumen fluid pH. On the other hand, this conclusion is partially agreed with our findings in which reported the acetate to propionate ratio were significantly higher for lambs from traditional and TMR2. This result is inconsistent with the findings of Ma *et al.* (2014) and (2015) and Carro *et al.* (2000), who reported a lower acetic to propionic acid with feeding high concentrate based diets with lower NDF intake. Furthermore, a significantly lower

butyric acid was also reported in this study for lambs fed TMR1, lower NDF intake, which is also inconsistent with those reported by Ma *et al.* (2014). As general trend, the relative concentration of different volatile fatty acids in the rumen is influence by the concentrate part digestibility and the forage NDF (Allen *et al.*, 2009).

The ruminal ammonia concentration in the rumen fluid is very crucial for microbial protein synthesis and the minimum concentration required for optimizing feed digestion is 5 mg/dl (NRC, 2000) and concentration above 20 mg/dl is required for maximizing feed intake and digestibility (Yusuf *et al.*, 2013). The ruminal concentration of  $\text{NH}_3\text{-N}$  in the rumen fluid were not significantly ( $P > 0.05$ ) differ between treatments, but a lower values were reported for lambs fed very high NDF (TMR3) compared with all other groups, but all values within the required levels for proper feed intake and microbial growth. This result agreed with Manatbay *et al.* (2014), who reported that feeding ruminants low forage to concentrate ratio significantly increase  $\text{NH}_3\text{-N}$  and total volatile fatty acids. In contrast, Ma *et al.* (2015) reported a significantly lower  $\text{NH}_3\text{-N}$  with high fermented carbohydrate and increase with high NDF intake which completely disagreed with our finding. The low  $\text{NH}_3\text{-N}$  may be the result of reduced deamination process in the rumen and enhanced ammonia utilization by microorganisms (Hristov *et al.*, 2005). In addition, Chen *et al.* (2015) reported that forage to concentrate diets did not affect rumen nitrogen degradability with any protein supplement which disagreed with our results. The results of this study revealed that the change in rumen pH, caused by dietary regimes, affect the rumen ammonia levels by influencing the absorption and incorporation of ammonia in microbial mass.

The biochemical indicators are very crucial to specify the feeding status and nutrients supply adequacy, as well as to prevent health disorders and consequently productivity performance. Different feeding regimens with varied fiber and non-fibrous carbohydrates levels affect the concentration of many blood metabolites of animal body, especially the ones influence the energy metabolism. All the metabolites values were fallen within the normal range reported by Jackson and Cockcroft (2002). High glucose level is mainly attributed to the high ruminal propionate level. The high level of glucose in the blood of lambs from the traditional, traditional pelleted and TMR3 may be caused by altering the metabolic process including decreased use of glucose with increased oxidation of butyrate and ketogenesis (Giesecke *et al.*, 1979; Baldwin and Jesse, 1992). The concentration of butyric acids in the rumen fluids of lambs from TMR1 group were very low compared with other groups which may support the previous hypothesis related the low glucose levels even

though propionate levels was high. This means that butyric levels in the rumen fluid may play an important role in glucose and energy metabolism.

## CONCLUSION

Feeding growing lambs with total mixed rations contains around 30% of NDF would be lead to obtain a suitable rumen characteristics, microbial fermentation, growth performance and consequently higher profit compared to a traditional feeding with alfalfa hay and barley. Moreover, pelleting of alfalfa and barley did not cause any negative effect on the growing performance, but affect rumen tissues' color to be darker. The NDF is a critical ingredient of the ruminants' diets for healthy and efficient productivity.

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### Statement of conflict of interest

Authors have declared no conflict of interest.

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