Pakistan J. Zool., vol. 49(4), pp 1519-1522, 2017. DOI: http://dx.doi.org/10.17582/journal.pjz/2017.49

Short Communication

Effect of Alfalfa Hay on Rumen Fermentation Patterns and Serum Biochemical Profile of Growing Naemi Lambs with *Ad Libitum* Access to Total Mixed Rations

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Article Information
Received 24 July 2016
Revised 24 August 2016
Accepted 18 October 2016
Available online 23 June 2017

Authors' Contributions
MMA, IA and AHA designed the study; RUK edited and revised the paper and ARSB, MYAS and RAA conducted the study and analyzed the samples.

Key words
Total mixed rations, Alfalfa hay, Growing lambs, Rumen characteristics.

ABSTRACT

Total mixed rations (TMR) based diet is a high energy diet, which negatively deteriorates the rumen health through pH reduction .This study investigated the effect of different protocols of alfalfa hay supplementary on ruminal characteristics and performance of growing Naemi lambs fed TMR. Four groups, each of 3 months old growing Naemi male lambs (28.85±1.09 kg), were fed on TMR (NDF=41. 95%) (T1). TMR plus 100 g alfalfa hay (NDF= 43.3%) daily (T2), TMR plus 200 g alfalfa hay (NDF= 43.3%) every two days (T3) and TMR plus 300 g alfalfa hay (NDF= 43.3%) every three days (T4). Rumen fluid samples were analyzed for volatile fatty acids and ammonia nitrogen levels. Acetic acid improved significantly in experimental groups, while propionic acid decreased significantly in T1. Valeric acid and butyric acid decreased significantly in T1 and T2 while isovolaric acid and the ratio of acetic acid and propionic acid and glucose increased significantly in T1. In conclusion, feeding TMR with alfalfa hay, regardless of the supplementary protocol, causes a significant change in rumen fermentation patterns as a result of increasing the intake level of NDF from the alfalfa hay. This shift in the fermentation pattern may positively affect the rumen environment and consequently growing lambs' health and productivity.

Lamb fattening in the Middle East and Saudi Arabia has significantly contributed towards red meat production (Alhidary *et al*., 2016 a, b). The in vogue traditional fattening system of lambs is very costly and may not cover the lambs' nutrient requirements to obtain their genetic potential. During the last few years, the traditional feeding system in Saudi Arabia has moved to be an intensive system using total mixed ration (TMR) in order to increase and maximize animals' productivity. Additionally, Ministry of Agriculture (MOA) and Ministry of Water and Electricity (MOWE) developed a new agriculture strategy to stop the expansion of high consuming water crops mainly forages for livestock. Feeding high concentrate and energy diets can potentially enhance feed efficiency and better productivity (Alvarez-Rodriguez *et al*., 2012; Tufarelli *et al*., 2013; Blanco *et al*., 2015; Cavini *et al*., 2015).

On the other hand, feeding TMR with high fermentable carbohydrate may increase the risk of subacute ruminal acidosis (Krause and Comba, 2003; Tufarelli *et al*., 2011) by depressing the ruminal pH and consequently affect ruminant animal health and productivity. The negative impact of low rumen pH may potentially decrease the digestibility of dry matter, acid detergent fibre (ADF), nutrient detergent fibre (NDF) and volatile fatty acids with lower acetic acid and higher propionic when compared with normal rumen pH (Calsamiglia *et al*., 2002). The risk of ruminal acidosis can be reduced by adopting feeding regime and using efficient buffering system (Santra *et al*., 2003; Tripathi *et al*., 2004).

It has been well documented that forages enhances the feeding, chewing activity, rumination, rumen motility, stimulate rumen size and support rumen epithelium and papillae which has a positive impact on digestion and assimilation (Tufarelli *et al*., 2009; Alvarez-Rodriguez *et al*., 2010). The NDF in the plants is the major nutritional components of roughages which is responsible for chewing and saliva production (Michael, 1997). Beauchemin and Buchanan-Smith (1989) reported that increasing NDF increased ruminating and chewing time. Alfalfa is important hay in ruminant nutrition due to its excellent

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0030-9923/2017/0004-1519 $ 9.00/0

nutritional values, including digestible protein, vitamins and minerals. Moreover, alfalfa hay is a main source of NDF that has a capacity to promote ruminal contractions, ruminal net formation and salivation and hence maintain appropriate rumen pH.

Therefore, the objective of this study was to find the effect of increasing levels of NDF (41.95 *vs.* 43.3%) using alfalfa in the diet of Naemi lambs fed at different protocols on rumen volatile fatty acids and blood biochemical profile.

Materials and methods

Growing male Naemi lambs (n= 40), 3 months old, were used in this experiment. The average body weight was 28.85±1.09 kg at the start of the experiment. Healthy lambs were housed in an individual pen at the Research Station, King Saud University (KSU).

Table I.- Chemical composition of total mixed rations and alfalfa (%, on dry matter basis) (mean ± SD).

Ingredients (dry matter basis, %)	Total mixed rations	Alfalfa
Barley	18.00	-
Wheat	29.92	-
Palm Kernel Meal	20.00	-
Soya Hulls	12.03	-
Wheat Bran	3.00	-
Alfalfa	6.00	-
Salt	0.47	-
Limestone	2.58	-
Molasses	7.85	-
Commercial Premix*	0.15	-
Composition (%)		
Dry matter	91.04±1.1	18.1±0.03
Crude protein	12.4±0.3	18.5±0.8
Crude fibre	11.98±0.12	28.2±1.2
Ether extract	2.61±0.17	1.9±0.03
Neutral detergent fibre	41.95±2.11	43.3±2.13
Acid detergent fibre	26.10±3.42	21.6±0.8
Ash	9.09±0.4	10.95±0.1

*Contained per kg, 10000 IU vitamin A, 1000 IU vitamin D, 20 IU vitamin E, 300 mg Mg, 24 mg Cu, 0.6 mg Co, 1.2 mg I, 60 mg Mn, 0.3 mg Se, 60 mg Zn.

The lambs were randomly divided after 15 days of the adaptations period to 4 dietary treatments (10 lambs/treatment). The dietary treatments were: control (TMR), control + 100 g alfalfa per day (T1), control + 200 g alfalfa per 2days (T2), control + 300 g alfalfa per 3 days (T3). The ingredients and chemical composition of the experimental diets is given in Table I. The experimental diets was offered early in the morning at 0800 h. The average NDF of control and experimental groups was 41.95% and 43.3%, respectively. Lambs were fed these diets *ad libitum* for 84 days. Feed samples were analyzed for nutritional contents on weekly basis. All lambs were bled every 4 weeks via the jugular vein. At the end of the experiment, blood samples were centrifuged and serum was separated (AOAC, 1990) and stored at –20ºC until analysis.

The rumen fluid samples were analyzed for acetic, propoininc, butyric, valeric and isovaleric using Gas chromatography (Nukol, Supelco™ WSAF-2 Mix from SUPLECO CO, Bellefonte, PA). The rumen fluid NH_3-N concentration was measured using a 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). The concentration of ammonia was directly proportional to the absorbance of indophenol (Broderick and Kang, 1980). Moreover, serum samples were analyzed for calcium and phosphorus, glucose, triglyceride, total protein, urea-N and cholesterol concentrations using commercial kits (Biocheck, USA) by the Spectrophotometer (Perkin Elmer, Waltham, MA, USA).

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the 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.

Results

The effect of feeding TMR alone or with different levels of alfalfa hay to growing Naemi lambs on the volatile fatty acids and ammonia-N concentrations in the rumen fluid are shown in Table II. A significantly (P<0.05) higher acetic acid levels were reported in lambs in T1, T2 and T3 compared to the control group. Propoinic acid was significantly low (P<0.05) in T1 compared to the control. Butyric and valeric acids were significantly lower in T1 and T2 while isovaleric acid was significantly lower in T2 and T3. The ratio of acetate and propionate and ammonia concentration was significantly higher in T3.

Data regarding the serum concentrations of biochemical variables of growing Naemi lambs are shown in Table III. There was no significant effect (P>0.05) of feeding TMR alone or with different alfalfa hay protocols on the total protein, urea N, cholesterol, triglyceride, calcium and phosphorus. The blood glucose was significantly (P<0.05) higher in lambs of T1 and T2 compared to the control.

Discussion

As a general trend, feeding high NDF in roughages slows down the rumen fermentation pattern and lowers the volatile fatty acid production with a high proportion of acetate to valerate and high acetate to propionate ratio in the rumen fluids (Carro et al., 2002). Feeding concentrate

Table II.- Effects of feeding growing lambs TMR alone or with different protocols of alfalfa hay supplement on the rumen concentrations of volatile fatty acid (g fatty acid/ 100 g total VFAs) and ammonia-N (mg/dl) of the growing Naemi lambs.

Treatment	Acetic acid	Propionic acid	Butyric acid	Valeric acid	Isovaleric acid	Acet:prop	Ammonia-N
Control	25.59[b]	41.21[a]	15.86[a]	3.52[a]	3.82[a]	0.62[a]	24.18[a]
T1	34.95[a]	29.46[c]	27.57[b]	2.10 [b]	5.92[b]	1.18[c]	22.12[a]
T2	32.30[a]	36.45[b]	26.02[b]	2.48[b]	2.75[c]	0.89[b]	16.89[b]
T3	30.05[a]	45.69[a]	18.30[a]	3.75[a]	2.21[c]	0.66[a]	13.28[c]
Pooled SE[2]	1.04	2.04	1.45	0.25	0.53	0.01	2.01

Different superscripts in a column carrying different letters are significantly different (P<0.05); Control, TMR; T1, TMR+ 100 g alfalfa hay/day; T2, TMR+ 200 g alfalfa hay/ 2days; T3, TMR+ 300 g alfalfa hay/ 3 days.

Table III.- Effects of feeding growing lambs TMR alone or with different protocols of alfalfa hay supplement on blood serum metabolites of the growing Naemi lambs.

Treatments/ metabolites	Control	T1	T2	T3	Pooled SE
Glucose (mg/dl)	65.16[a]	77.66[b]	71.86[c]	65.66[a]	0.10
Total Protein (g/l)	63.45	59.63	60.66	62.54	2.35
Urea (mg/dl)	16.36	18.15	20.21	15.46	1.10
Cholesterol (mg/dl)	83.78	88.34	96.58	89.86	3.52
Triglyceride (mg/dl)	43.36	42.96	41.08	50.54	2.32
Ca (mg/dl)	8.88	10.21	9.32	9.56	0.56
P (mg/dl)	8.54	8.12	9.11	8.32	0.23

Different superscripts in a column carrying different letters are significantly different (P<0.05). For details of experimental groups, see Table II.

based diet for 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 conclusion completely agrees with our findings in which lambs fed TMR (C) and TMR with 300 g alfalfa every 3 days showed a significantly higher propionic and valeric with lower butyric acids compared with T1 and T2 lambs. Moreover, the proportions of acetic to propionic acids reflect the rumen fermentation patterns and microbial fermentation efficiency. High dietary fiber increases the acetic acid and high propionate with high starch fermentation and lower pH which may lead to acidosis. In this study, the proportion of acetic to propionic acids was significantly higher in lambs in T1, T2 and T3 fed with higher NDF compared with the control. This result is consistent with the findings of Ma *et al.* (2014), (2015), and Carro *et al*. (2002) 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 lambs fed TMR (C) and from T3, which is inconsistent with Ma *et al*. (2014). It is very clear from the fermentation pattern and volatile fatty acid production of lambs from the control and T3 following the same trend even though high NDF intake every three days.

The ruminal concentration of NH_3-N differed significantly between treatments with high value in lambs fed TMR and fed 100 g alfalfa hay daily (T1) compared with T2 and T3. This result agreed with Manatbay *et al*. (2014) who reported that feeding low forage to concentrate ratio significantly increased NH_3-N and total volatile fatty acids. In contrast, Ma *et al*. (2015) reported a significantly lower NH_3-N with high fermented carbohydrate and increase with high NDF intake which completely disagreed with our finding. The low NH_3-N may be the result of a reduced deamination process in the rumen and enhanced ammonia utilization by microorganisms (Hristov *et al*., 2005). Recently, 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, affects 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 nutrient supply adequacy, as well as to prevent health disorders and consequently productivity performance (Alhidary *et al*., 2016a). Different feeding regimens with varied fiber and non-fibrous carbohydrate levels affect the concentration of many blood metabolites of the animal body, especially the ones influences the energy metabolism. The results of this study showed a significant difference (P<0.05) in glucose level, which was lower in lambs in the control and T3 compared to the lambs in T1 and T2. Moreover, there was no significant effect (P>0.05) of feeding growing lambs TMR alone or with different alfalfa hay feeding protocols on the total protein, urea N, cholesterol, triglyceride, calcium and phosphorus. The values of all the metabolite fell within the normal range reported by Jackson and Cockcroft (2002). This result is very difficult to be explained since it is well-known that the high glucose level is mainly attributed to the high ruminal propionate level. The high level of glucose in the blood of lambs in T1 and T2 may be caused by altering the metabolic process such as reduced uptake of glucose or increased oxidation of butyrate (Giesecke *et*

al., 1978; Baldwin and Jesse, 1992). The concentration of butyric acids in the rumen fluids of lambs in T1 and T2 was very high compared with other groups which may support the previous hypothesis related the high glucose levels of these groups since propionate levels were low.

Conclusion

From the results of the present study, we concluded that feeding TMR with alfalfa hay, regardless of the supplementary protocol, cause a significant change in rumen fermentation patterns as a result of increasing the intake level of NDF from the alfalfa hay.

Acknowledgement

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group No RG- 1436-021. Special thanks to Dr. Gamal Suliman, Eng. Rafat Mahmoud and Dr. Abdelrahman Jaralnabi for their helps in samples collection, preparation and analysis.

Statement of conflict of interest

The authors declare no conflict of interest regarding this paper.

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