Pakistan J. Zool., vol. 54(6), pp 2881-2887, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20211029041056

Effect of Artificial Saliva Supplementation on Growth, Rumen Characteristics and Fecal Odor of Growing Naeemi Lambs Fed Total Mixed Ration

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Article Information
Received 29 October 2021
Revised 07 November 2021
Accepted 24 November 2021
Available online 03 March 2022 (early access)
Published 29 August 2022

Authors' Contribution
MA, IH and MSA designed and outlined the experiment, wrote the manuscript and follow up of the feeding trial. RJ assisted in data collection and statistical analysis, and reviewed the article. MO execute the experiment and analyzed the samples. RUK edited the paper. All authors critically reviewed the data, wrote and revised the final version of the manuscript.

Key words
Artificial saliva, Growing lambs, Iron, Rumen color, Rumen pH

ABSTRACT

We investigated the effect of feeding a complete feed as a total mixed ration (TMR) with different levels of artificial saliva (AS) on the rumen pH, epithelial tissues pigmentation, fecal odor and status of some trace minerals. Forty-five growing lambs were used in an 84-day trial. Lambs were randomly assigned to five dietary treatments as follows: TMR, AS1 (TMR +15 kg AS/Ton; 22.5 g/lamb/day), AS2 (TMR + 30 kg AS/Ton; 45 g/lamb/day), AS3 (TMR + 45 kg AS/ Ton; 67.5 g/lamb/day) and AS4 (TMR +90 kg AS/Ton; 135 g/lamb/day). The growth performance was not affected by the supplementation of AS. The addition of AS improved the rumen color and maintained the rumen pH of the lambs. The fecal H_2S gas significantly ($P<0.05$) higher in AS4 group compared to the TMR. However, serum iron (Fe) content was significantly ($P<0.05$) higher in TMR fed group alone. From the findings of the present study, we concluded that AS supplementation improved rumen color, however, fecal H_2S production was increased which needed to be further investigated.

INTRODUCTION

Feeding a full feed as a total mixed ration (TMR) containing highly fermentable carbohydrate (CHO) may raise the risk of subacute rumen acidosis by lowering ruminal pH, compromising ruminant animal health and production (Alhidary *et al*., 2016a; Alharthi *et al*., 2021a, b). TMR fibre stimulates rumination, chewing activity, and saliva production, which helps to buffer rumen pH and maintain an ideal environment for rumen bacteria (Alhidary *et al*., 2016b). Feeding TMR low in fiber content and small particle size will lead to decreased chewing activity and salivary production, ultimately lowering the rumen pH. Moreover, it is used to determine the chemical and physical characteristics of roughage as a source of effective fiber (Lee *et al*., 2004). The negative or positive effects of feeding complete feed may be caused by the levels used and the chemical composition of the ingredients, especially when byproducts and premixes are used. For this reason, special consideration must be given to using byproducts due to their negative effects, especially when used at high levels. For example, the dark-brownish color of rumen epithelium tissues may be caused by many factors including high iron intake, assuming that the pigmentation is an iron-flavonoid complex. Alhidary *et al*. (2016a) reported that TMR plus alfalfa can affect the production of a pigment that attaches firmly to the keratinized confirmed layer of the rumen epithelium tissues. The risk of rumen acidosis (low pH) can be reduced by controlling the rumen fermentation process by feeding high fiber or using feed additives such as artificial saliva (AS), which are designed to reduce the negative effects of feeding highly fermented CHO on rumen characteristics.

Salivary secretions are considered to be the primary natural buffering system that maintains rumen function by controlling the rumen environment, especially rumen pH. In addition, saliva plays an important role as a fluid medium for feed mastication, swallowing, and transport of ingesta through the rumen and reticulum. Krause and Oetzel

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0030-9923/2022/0006-2881 $ 9.00/0

(2006) and Jouany (2006) reported that the normal range of ruminal pH of 6.0–7.2 is the optimum environment for rumen microbial activities, which are mainly regulated by saliva. Thus, AS may play an important role in controlling rumen pH in cases of unbalanced rations and low NDF dietary levels. AS is produced in the form of powder containing a mixture of chemical compounds ($NaHCO_3$, KCl, $CaCl_2$, $Na_2HO_4.12H_2O$, NaCl, and $MgSO_4.7H_2O$) in appropriate proportions (McDougall, 1948).

The objective of this trial was to study the effects of feeding four levels of AS on the rumen characteristics, trace minerals, and fecal odor status of growing lambs fed complete feed as a TMR.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Ehtical Committee on Animal Rights, King Saud University, Riadh, Saudi Arabia.

Animals and study design

A total of 45 growing lambs (25±3 kg average body weight; 90 days old) were used in an 84-days trial. Lambs were weighed, ear tagged, vaccinated against clostridial diseases, and treated for internal and external parasites. Thereafter, the lambs were distributed randomly to group feeding pens, with three lambs per pen for fourteen days adaptation period for the new diets. On day 1 of the experimental period, the pens were randomly assigned to one of the five dietary treatments (nine lambs per treatment on the basis of weight). The dietary treatments were as follows: TMR, AS1 (TMR+15 kg AS/Ton, 22.5 g/lamb/day), AS2 (TMR+30 kg AS/Ton, 45 g/lamb/day); AS3 (TMR+45 kg AS/ Ton, 67.5 g/lamb/day) and AS4 (TMR+90 kg AS/Ton, 135 g/lamb/day). Aartifical saliva is produced in a form of powder containing a mixture of chemical compounds ($NaHCO_3$, KCl, $CaCl_2$, $Na2HO_4.12H_2O$, NaCl, and $MgSO_4.7H_2O$) in appropriate proportions.

Performance traits

The weights of offered feed and feed refusals were measured weekly, and the feed intake was calculated on a dry matter basis per pen. Lambs were weighed before morning feeding at 0730 h on day 1 of the study and at the end of the study. The gain to feed ratio for each lamb was calculated and expressed as body weight gain per kg of dry matter intake.

Serum minerals

Blood samples were collected from each lamb via jugular venipuncture at the end of the study. Serum was obtained by centrifugation at 2400 × g for 15 min at 4 °C. Serum trace mineral concentrations were analyzed at the beginning and the end of the trial using ICP-MS (Inductively Coupled Plasma-Mass Spectrometer: Perkin Elmer, USA).

Rumen pH

The pH values of ruminal fluid from three lambs/ treatment were measured at 30-min intervals for 60 d using rumen pH/temperature sensor (Horiba Twin pH, Spectrum Technologies, Inc. 60544, Plainfield, IL, USA) , and measurements were stored using a data logger and analyzed after downloading. The pH value of ruminal fluid was recorded every 30 min, and hourly means were calculated. Samples for rumen pH were taken on weekly basis after three h of feeding the AS. The average values for the whole experimental perios of each treatment were compared.

Rumen color

At the end of the trial, four lambs from each treatment were randomly slaughtered, and rumen tissue samples were collected from the dorsal sac and used for color measurement. The color of rumen tissues was determined using a Minolta Chroma Meter (Konica Minolta, CR-400, Japan) with a CIELAB Color System for the color values (L* = value designates lightness).

Fecal odor gases

Before the end of the feeding trial, three lambs were selected from each treatment for the determination of fecal odor gases. Feces and urine were collected daily for 4 d and mixed well, and then homogenized samples were taken. Fecal samples were mixed with urine in a glass tube (50 g feces with 25 mL urine; 12 tubes per treatment). Gas concentrations were measured using Gastec detector tubes designed for H_2S, NH_3, and acetic acid (Gastec Corp., Kitagawa, Japan).

Statistical analysis

All data was analyzed as a Complete Randomized Design with repeated measurements using the PROC Mixed and GLM Models of SAS. Means were separated using Tukey test. Significant differences were considered at $P < 0.05$.

RESULTS

Feed intake, body weight gain, and feed conversion ratio

Table I shows the effect of treatments on the growth performance and feed efficiency of growing Naeemi lambs fed different levels of AS. Lambs fed TMR and AS had no significant effect on body weight gain, feed intake and FCR.

Rumen fluid pH

Table II shows the rumen pH of the TMR and the treatment groups. As a general trend, addition of AS at all levels increased (P > 0.05) the rumen pH compared with TMR.

Table I. Effect of dietary different levels of artificial saliva on the performance of growing lambs fed complete feed as TMR.

Measurements treatments	TFI (Kg)	BWG (Kg)	FCR
AB	140.51a	18.08b	7.94a
TMR	142.59a	26.83a	5.32b
AS1	133.31d	22.14c	6.03b
AS2	135.23d	22.07c	6.15b
AS3	129.70b	21.54c	6.04b
AS4	125.73b	19.44b	7.29a
SEM	1.86	0.74	0.23
P value	0.001	0.000	0.000

AB, traditional feeding; TMR, Total mixed ration; AS1, artificial level 1; AS2, artificial level 2; AS3, artificial level 3; AS4, artificial level 4. TFI, total feed intake; BWG, body weight gain and FCR, feed conversion ratio; SEM, Standard error of the means. Column with different superscripts with small letters show significant differences

Table II. Time distribution throughout the day of rumen pH changes for lambs fed artificial saliva.

pH	Traditional AB	TMR	T1 AS1	T2 AS2	T3 AS3	T4 AS4
less than 5.3	0	870	0	0	0	0
5.3–5.8	0	570	15	1065	0	968
5.8–7	1440	0	1425	375	1440	473

For abbreviations, see Table I.

Rumen color

The effects of treatment on the color of rumen tissues are presented in Table III. As a general trend, feeding all AS levels caused an improvement in color values (L values) compared with TMR alone. The rumen tissue color of lambs from the AS1 group showed a significant (P < 0.05) improvement in color values compared with TMR and other levels of AS2, 3, and 4.

Fecal odor

Regarding the unacceptable fecal odors linked to acetic acid, H_2S, and NH_3 emis-sions, there was no significant effect of treatment on acetic acid emission levels, but sig-nificant differences were reported for H_2S emissions as a result of the treatments. A sig-nificantly lower fecal H_2S level was reported for lambs fed TMR, and a higher level was reported for lambs from AS4. Furthermore, for NH_3 levels, no significant differences were found between the control and the treatment groups (Table IV).

Table III. Minimum, maximum, and average rumen fluid pH during the day in treatments supplemented with artificial saliva.

	Traditional AB	TMR	T1 AS1	T2 AS2	T3 AS3	T4 AS4
Mean	6.05	5.28	5.88	5.68	6.05	5.70
Minimum	4.85	4.66	5.19	4.72	4.91	4.94
Maximum	7.01	6.62	6.38	6.82	6.90	7.18
ST.V	0.41	0.33	0.18	0.42	0.32	0.47

For abbreviations, see Table I. ST.V, standard of variation.

Table IV. Effect of dietary different levels of artificial saliva on fecal odor gases of growing lambs fed complete feed as TMR.

Measurements treatment	Acetic acid (ppm)	H_2S (ppm)	NH_3 (ppm)
AB	1.69	31.25b	110.00
TMR	1.63	151.25c	50.00
AS1	1.05	96.25c	106.25
AS2	1.19	190.00c	81.25
AS3	1.56	360.00d	60.00
AS4	2.00	782.50a	50.00
SEM	0.17	22.00	8.85
P value	0.650	0.010	0.149

For abbreviations, see Table I. SEM, Standard error means. Columns with different superscripts with small letters showed significant differences.

Trace elements

Table V shows the effect of the treatments on trace minerals concentrations in the serum of the experimental lambs. The results showed a significant difference between all treatment groups in terms of Fe concentrations. Fe concentration was significantly (P <0.05) higher in TMR compared to the treatments.

DISCUSSION

Feeding ruminant animal complete feed with high fermentable carbohydrate increases the levels of ruminal organic acids such as volatile fatty acids (VFAs) and lactic acid accumulation, which may cause acidosis by decreasing pH. As a consequence, the pH of the rumen drops. Ruminal pH levels of 5.6 and lower are used to characterize subacute ruminal acidosis (SARA) and ruminal acute acidosis (RAA) (Nagaraja and Tigemeyer, 2007). Immediately after feeding, rumen pH normally drops, but it causes problems when it is low for more than 3 h. This can have a negative effect on animal health and

Table V. Effect of dietary different levels of artificial saliva on blood serum mineral concentrations of growing lambs fed complete feed as TMR.

	Treatments							
Trace elements	AB	TMR	AS1	AS2	AS3	AS4	SEM	P value
Mn, $\mu_{g/L}$	2.95[b]	4.13[a]	4.10[a]	4.02[a]	3.76[a]	4.31[a]	0.13	0.001
Fe, $m_{g/L}$	1.32	1.40	1.43	1.48	1.34	1.50	0.02	0.23
Co, $\mu_{g/L}$	0.42	0.48	0.49	0.48	0.47	0.48	0.01	0.15
Cu, $_{mg/L}$	1.13	0.93	1.00	0.96	1.02	1.48	0.08	0.60
Zn, $_{mg/L}$	0.58[a]	0.68[b]	0.77[b]	0.71[b]	0.67[b]	1.02[a]	0.04	0.02
Se, $\mu_{g/L}$	112.58	129.61	151.67	120.89	134.93	157.36	7.33	0.54

For abbreviations, see Table I. [a, b] Means with different superscripts within each row were significantly different. SEM, Standard error of means.

productivity and cause dark coloration of the rumen epithelial tissues (Shusterman, 1992). This coloration may also be caused by a high intake of iron from plant byproducts such as PKC, which contain high levels of minerals, especially iron (Alimon, 2004) and phenols. The iron reacts with phenols when the pH drops in the rumen fluid to form a blackening ferric-polyphenol compound (Hamada *et al*., 1969), which may lead to discoloration of the rumen epithelial tissues. The complete feed confirmed the formation of black stain in the rumen tissues, which makes it unacceptable to consumers (Hamada *et al*., 1969; McGuire *et al*., 1985). On the other hand, incomplete microbial degradation of dietary nutrients such as protein and carbohydrates in complete feed as a result of pH decreases which may increase odorous compounds in the manure (Zhu and Jacobson, 1999). These can harm human health and cause environmental pollution. This trial examined the effect of using AS as a rumen buffering feed additive to control rumen pH and avoid acidosis, and consequently to enhance the rumen microbial fermentation process to reduce fecal odors.

In this study, the lambs fed TMR had pH values in the rumen fluid as follows: 5.3 for 870 min after feeding and from 5.3 to 5.8 for 570 min per day, with a mean value of 5.28 (Tables II, III). This can be linked to the very dark color in rumen tissues (L = 29.1). The addition of AS caused an increase in pH values compared to the TMR group and improved rumen tissue color. This result confirms that feeding TMR can reduce rumen fluid pH and form blackening ferric-polyphenol compounds that result from the inclusion of palm kernel meal in the complete feed. As a general trend, the addition of AS at all levels increased the rumen pH compared with TMR, but was almost the same as that of the AB group.

Furthermore, feeding all AS levels caused an improvement in color values (L values) compared with TMR alone, but was lower than that of rumen tissues in the AB group. The rumen tissue color of lambs from the AS1 group showed a significant ($P < 0.05$) improvement in color values compared with TMR and other levels of AS2, 3, and 4, but tissue from AB lambs showed a significantly brighter coloring compared with the other groups. The darker color, lower rumen fluid, and other performance measures of growing lambs fed TMR consisting of PKC compared with traditional feeding have been reported in several recent studies (Alhidary *et al*., 2016a, b, 2017; Abdelrahman *et al*., 2017, 2019). In order to reduce serious health disorders caused by rumen acidosis that can negatively affect ruminant animal performance, it is crucial to search for a good alternative to improve rumen pH (Aschenbach *et al*., 2011; Owens and Basalah, 2016). Different commercial buffering additives (Mao *et al*., 2017) have been introduced to the market to help with maintenance of proper rumen pH levels. These include AS, sodium bicarbonate, calcified seaweed, and malic acid, which can be added to TMR (Alhidary *et al*., 2017; Abdelrahman *et al*., 2019). A recent study conducted by Alhidary *et al*. (2019), supported our finding that the addition of different buffers to TMR of growing lambs can improve their growth performance, rumen pH, fermentation process, and meat quality. Furthermore, Abdelrahman *et al*. (2019) conducted an experiment using malic acid as a rumen buffering agent in TMR consisting of palm kernel meal for growing lambs and measured its effect on rumen characteristics. They concluded that malic acid caused a significant improvement in rumen pH and rumen tissue coloring by decreasing the lactic acid accumulation compared with regular TMR. These findings are in agreement with the results of this trial. The main problem with using different buffering agents is determining the proper level that can be used without negative effects on ruminant animal health and productivity.

On the other hand, a higher accumulation of iron in the rumen tissues of lambs fed TMR, AS2, and AS4 compared with those of AB, AS1, and AS3, which may indicate that high iron intake and accumulation in the rumen tissues resulting from the formation of ferric-polyphenol compounds play a crucial role in dark coloring, in addition to other factors. Regarding the trace mineral status of growing lambs as a result of feeding TMR with or without AS at different levels, feeding lambs complete feed improved blood serum trace mineral levels compared with traditional feeding because of the well-balanced mineral additives in the complete feed. There was no significant effect of the treatment on trace mineral levels in the blood serum, except for Mn and Zn. All of the trace mineral values were within normal levels according to Puls (1990). Moreover, the Mn and Zn levels in the blood serum of lambs from the AB group were significantly lower than those in the other dietary groups (Table V).

Fecal gas odors are an acute environmental problem (Singh and Rashid, 2017) and have an adverse effect on human health and the environment (Ushida *et al*., 2003; Singh and Rashid, 2017). Manipulation of the rumen fermentation process by using feed additives is a potential solution for reducing emissions of these gases. In this study, the effects of using AS on the fecal unacceptable smells, which are linked to acetic acid, H_2S, and NH_3 emissions, were analyzed. There was no significant effect of treatment on acetic acid emission levels, but significant differences were reported for H_2S emissions as a result of the treatments. A significantly lower fecal H_2S was reported for lambs fed AB, but a higher value was reported for lambs from AS3 and AS4. Lambs from the TMR, AS1, and AS2 groups were not significantly different ($P > 0.05$; Table IV), but numerically lower values were found for lambs from the AS1 group. Furthermore, for NH_3 levels, no significant differences were found, but there was a trend of variation between all treatment groups. To our knowledge, no studies have investigated the effect of using AS on fecal gas emissions, especially in ruminant animals. The numerical differences detected between all treatment groups in term of fecal gases gives a general indication of improvement as a result of using AS as a feed additive to improve nutrient digestibility, especially protein and carbohydrate.

CONCLUSION

AS supplementation with complete feed as a TMR improved rumen fluid pH and rumen tissue coloring, but a negative effect on the growth performance of lambs was found at high levels of AS supplementation. Moreover, AS1 and AS3 supplementation reduced the iron accumulation in rumen epithelial tissues, which may indicate that high iron intake plays a role in dark rumen pigmentation by forming blackening ferric-polyphenol compounds, in addition to other possible factors that are not yet well-defined. Including Level 1 AS (15 kg/ton: 22.5 gm/ lamb/day) is highly recommended to improve the rumen fermentation process by increasing rumen pH value to >5.8. Moreover, this can reduce the dark pigmentation of rumen epithelial tissues and fecal NH_3 emissions.

ACKNOWLEDGMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group RG -1441-408.

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