EFFECT OF LEVEL OF CONCENTRATE FEEDING AND ADDITION OF MONENSIN ON RUMEN FERMENTATION CHARACTERISTICS IN AWASSI LAMBS

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Abstract

This study was conducted to investigate the effect of level of concentrate feeding and addition of monensin on fermentation characteristics of Awassi lambs. 16 Awassi male lambs were used with 4-6 months of age and mean initial weight of 21.27 kg. concentrate diet was offered at 2 levels (2.5 and 3% of BW) with or without addition of monensin at rate 30 mg/kg DM, ground wheat straw was offered *ad libitum*. Results showed that increasing level of concentrate increased ($P<0.01$) NH_3-N concentration from 6.59 to 7.13 mg/100 ml without affecting TVFA concentration which was significantly decreased ($P<0.01$) from 14.14 to 12.10 mmol/100 ml as a result of addition of monensin at rate of 30 mg/kg DM. Despite of slight increase, pH values were not significantly affected by both factors. Concentrations of NH_3-N and TVFA showed significant response ($P<0.01$) to the interaction effect between those two factors. In the study of diurnal changes, rumen fermentation characteristics showed an expected responses to the time of withdrawing rumen fluid samples from lambs.

Key word: monensin, concentrate, rumen fermentation,

تأثير مستوى تغذية العلف المركز واضافة المونسين في خصائص تخمرات الكرش للحملان العواسيه

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المستخلص

اجريت هذه الدراسة للتحري عن تأثير مستوى العلف المركز واضافة المونسين في خصائص تخمرات الكرش للحملان العواسية. استخدم في الدراسة 16 حمل عواسي ذكري. تراوحت اعمارها بين 4 الى 6 اشهر وبمتوسط وزن ابتدائي بلغ 21.27 كغم. قدم العلف المركز بمستويين (2.5 و3% من وزن الجسم) بدون او بإضافة المونسين بمعدل 30 ملغم/كغم مادة جافة فيما قدم تبن الحنطة المجروش بصورة حرة. اظهرت نتائج الدراسة الحالية ان زيادة مستوى العلف المركز ادت الى ارتفاع ($P<0.01$) تركيز نيتروجين الامونيا من6.59 الى 7.13 ملغم/100 مل دون التأثير على تركيز الاحماض الدهنية الطيارة الكلية الذي سجل انخفاضا معنويا ($P<0.01$) من 14.14 إلى 12.10 مليمول/100 مل نتيجة لإضافة المونسين بمستوى 30 ملغم/ كغم مادة جافة. وبالرغم من ارتفاعها الطفيف لم تتأثر قيم الاس الهيدروجيني معنويا بكلا العاملين. كما اظهر تركيز نيتروجين الامونيا والاحماض الدهنية الطيارة الكلية استجابة معنوية ($P<0.01$) بتأثير التداخل بينهما. وفي دراسة التغير الزمني اظهرت خصائص تخمرات الكرش استجابة متوقعة وفقا لزمن سحب نماذج سائل الكرش من الحملان.

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Introduction

Rumen fermentation is one of the particular factors determining the physiological aspects of ruminant digestion. Animal nutritionists have been interested in using additives to improve animal production by manipulating ruminal fermentation (17). The efforts have particularly focused on substances that increase fiber digestibility, ruminal propionic acid, saliva output and that reduce ruminal acidosis, bloat incidence, methanogenesis, ruminal proteolysis and deamination of dietary protein (32).

Carboxylic polyether ionophore antibiotics, produced by various strains of Streptomyces spp., are rumen metabolic modifiers including products such as monensin, lasalocid and salinomycin (33). Monensin are widely used as feed additives for ruminants to increase feed efficiency by influencing a ruminal fermentation.

It was well known that feeding concentrate diets accelerated ruminal fermentation leading to reduce ruminal pH. The lower pH after feed ingestion could be the result of feed fermentation in the rumen resulting in production of volatile fatty acids (VFA), hence lowering the ruminal pH (26). Scharen, et. al., (38) reported that monensin acts as a ruminal fermentation modifier by affecting the functional relationships among the ruminal microorganisms through reducing substrates availability by the inhibition of specific rumen microorganisms. Monensin improves cellulose digestion of high readily available carbohydrates diets by inhibiting the growth of lactate-producing bacteria, thereby decreasing lactate concentrations and increasing ruminal pH, passage rate, feed efficiency and daily weight gains (42). Safaei, et. al., (36) reported that monensin showed an improvement in food utilization and modulated fermentation in rumen by increasing propionate and decreasing acetate production. Therefore, this study aimed to evaluate the effect of introducing monensin in concentrate diet offered at low and high levels on rumen fermentation characteristics.

Material and methods

This study was carried out at the animal field/Animal Production Department- College of Agriculture- Al-Qasim Green University from 28/11/2018 to at 16/3/2019. The study included feeding Awassi lambs two levels of concentrate diet, 2.5 and 3% of BW with or without addition of monensin. Thus there will be 4 experimental treatments.

Concentrate diet was prepared by mixing several ingredients including, wheat bran, barley, yellow corn and soybean meal in addition to salt and minerals- vitamin mix. Level of each ingredients was estimated to ensure that concentrate diet will contain about 12.5% CP and 1.34 g of rumen degradable nitrogen (RDN) per each mega joule of metabolizable energy (g RDN/MJ of ME) (4). The estimated content of RDN was about 1.64 g/100 g DM and content of ME was about 1.23 MJ/100 g DM, then, the estimated ratio of RDN/ME was 1.34. The above estimations was performed according to the effective ruminal degradability of protein fraction in the ingredients used in preparation of concentrate diet as mentioned in table 1. Monensin as 10% monensin sodium was added at level of 30 mg/kg DM. Chemical composition of concentrate diet, its ingredients and wheat straw is shown table 1.

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Table (1). Chemical composition of concentrate diet, its ingredients and wheat straw (%)

Ingredients	DM	% in DM						ME MJ/100g
		Ash	OM	CP	CF	EE	NFE	
Wheat bran	91.75	5.48	94.52	14.27	13.96	3.77	62.52	1.23
Yellow corn	91.18	2.22	97.78	9.27	4.2	3.51	80.80	1.37
Barley	91.78	5.65	94.35	10.16	6.71	1.99	75.49	1.27
Soybean meal	91.93	7.87	92.03	45.48	3.75	1.83	39.35	1.18
Urea	-	-	-	287.5*	-	-	-	-
Concentrate	89.66	8.60	91.40	12.22	5.74	2.14	71.13	1.23**
Wheat straw	92.59	7.38	92.62	2.47	36.74	1.72	51.69	0.99**

*** 46% nitrogen × 6.25**

ME values were estimated according to MAFF equation (23) with subsequent conversion from MJ/kg DM to MJ/ 100 g DM in accordance with chemical composition based on percentage determinations:

****ME (MJ/ kg DM) = 0.012 CP +0.031 EE+0.005 CF +0.014 NFE**

NaCl and mineral-vitamin mix manufactured by …. Company were added to concentrate at rate of 1% for each. Urea was added at rate of 0.62% to ensure existence of a standard ratio of 1.34 g RDN/MJ of ME (ARC, 1984). Level of RDN was estimated according to previous studies in which the ruminal effective degradability of protein fraction in the different ingredients of concentrate diet had been determined as follows: 80 and 60% for barley and yellow corn respectively (19), 70% for soybean meal (2) and 67% for wheat bran (31).

Sixteen Awassi male lambs were bought from local market with an average initial weight of 21.275 ± 2.56 kg and 4 to 6 months of age were used in this study. Then lambs were randomly allotted into four treatments with 4 lambs per each and housed individually. About 4 weeks as preliminary period were passed before the beginning of the study for adaptation of lambs to individual cages and study conditions, during which, concentrate diets were offered gradually with two meals, 8 at morning and 4 at evening. Ground wheat straw was offered *ad libitum* to all lambs.

Rumen fluid was withdrawn from the experimental animals within one day of the tenth week at three times, before feeding, 3 and 6 hours thereafter. A plastic tube connected with a 50 ml veterinary syringe was used for collecting rumen liquid according to the method described by Saeed (35). The collected liquid was filtered through 4 layers of cheese cloth, then a few drops of 50% sulfuric acid solution were added to kill rumen microbes and stop fermentations. Samples of rumen liquid represented 3 withdrawal times for each animal were then saved in the freezer.

The pH of the rumen liquid was recorded soon after filtration of the withdrawn samples using Mi 180 Bench Meter pre calibrated with buffer solutions, the same technique was performed for rumen liquid drawn at the three times. Rumen fluid samples were then acidified as mentioned above, separated into two parts and each were placed in clean plastic tubes which were tightly closed and stored at – 20 C^{o}. The first frozen part of the rumen liquid was thawed and filtered by the centrifuge at 3000 rpm for 20 minutes to determine ammonia nitrogen concentration (NH_3-N) using the MgO distillation method (3). The second part of frozen rumen liquid samples were thawed and centrifuged at 300- rpm for 20 min. and used to determine concentration of volatile fatty acids (VFA) using method described by Markham (27). Data obtained was statistically analyzed according to factorial experiments (2 × 2) in completely randomized design (CRD) to evaluate the effect of the main factors studied in the experiment. Statistical Analysis System, SAS (37) was used for that purpose.

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Results and discussion

Main effect of level of concentrate and addition of monensin on fermentation characteristics

Effect of concentrate levels and addition of monensin on rumen fermentation characteristics of experimental diets is presented in table 2. As shown increasing level of concentrate had no significant effect on ruminal pH (6.88 vs. 6.93). Similar result was obtained by Fimbres, et. al., (14), they found that increasing level of concentrate from 3.4 to 4.5% of BW in the Pelibuey lamb diet had no significant on ruminal pH (6.4 vs. 6.5). In another study Azizi-Shotorkhoft, et. al., (7) found that increasing concentrate level in the Moghani lamb's diet had no significant on ruminal pH (6.02 vs. 6.23).

The lack of effect of increasing level of concentrate on ruminal pH may have associated with its insignificant effect on ruminal VFA concentration. The relation between these both parameters of rumen fermentation characteristics was reported by Huntington (20). Moreover, dietary inorganic matters are known for better absorption of VFA by removing unionized acid, and by exchange of ionized VFA during the absorption process and higher dietary ash content causes faster rate of passage, which aids in maintaining pH near neutrality (39).

Table 2- effect of level of concentrate and addition of monensin on fermentation parameter (as appeared in the table ± SE)

Fermentation parameter	Level of conc. % of BW		Monensin mg/kg conc.		*P*	
	2.5	3	0	30	Conc.	Mon.
pH	6.88 ±0.06	6.93 ±0.09	6.82 ±0.06	6.99 ±0.09	NS	NS
NH3-N, mg/100ml	6.59^{b} ±0.19	7.13^{a} ±0.10	6.75 ±0.23	6.97 ±0.10	**	NS
VFA, mM/100 ml	13.31 ±0.73	12.94 ±0.17	14.14^{a} ±0.42	12.10^{b} ±0.30	NS	**

Means in the same row with different superscripts are significantly different
**** (P<0.01) NS= Not significant**

Results also revealed that higher (P<0.01) ruminal NH_3-N concentration 7.13 as compared with 6.59 mg/100ml, was associated with increasing level of concentrate from 2.5 to 3% of BW. Consistently, Carro, et. al., (11) recorded an increase (P<0 05) in ruminal NH_3-N concentration from 20.7 to 24.3 mg/100 ml due to increasing concentrate level in the Merino sheep diet. In another study, Vosooghi-poostindoz, et. al., (41) observed that increasing concentrate level in the Kurdi lamb diet increased (P<0.05) ruminal NH_3-N concentration from 6.34 to 8.23 mg/100 ml.

The higher concentration of NH_3-N was mostly explained by the higher production of ammonia from the degradation of proteins in the rumen (22). This trend agreed with in vivo studies (12, 16).

Moreover, higher dietary concentrate level theoretically and practically increased CP intake which may increase ruminal NH_3-N concentration, then the difference in NH_3-N concentration could be related directly to CP intake. Dung, et. al., (13) reported that ruminal NH_3-N concentration may be affected by CP content and degradability. However, since concentrate diet in a current study was formulated to ensure well synchronization between energy and nitrogen levels then intake of dietary protein rather than its degradability was the critical

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factor involved in higher ruminal NH_3-N concentration.

Results also showed that ruminal TVFA concentration was insignificantly affected by increasing level of concentrate. This agrees with other findings demonstrating no effect of increasing level of concentrate on TVFA. Galvani, et. al., (15) found that ruminal VFA concentration was not significantly affected (6.87and 7.48 mM/100 ml) by increasing concentrate level in the ram lamb's diet. Insignificant effect of increasing concentrate level in the Pelibuey lamb's diet on VFA concentration in rumen liquid (2.28 vs. 2.63 mM/100 ml) was also obtained by Fimbres, et. al., (14).

As VFAs are the end products of rumen microbial fermentation and represent the main supply of metabolizable energy for the ruminant, insignificant effect on ruminal VFA concentration may suggests that increasing level of concentrate did not modify diet fermentability and energy availability (1). However, the slightly lower concentration of TVFA observed with higher level of concentrate in a current study may be attributed to higher absorption through the rumen wall as evidenced by slight increase in ruminal pH.

Statistical analysis of ruminal fermentation data pointed out that addition of monensin at rate 30 mg/kg DM had no significant effect on ruminal pH, Means were 6.82 and 6.99 for lambs fed concentrate diet without and with addition of monensin respectively. This result agrees with that obtained by Vendramini, et. al., (40) in which ruminal pH was not significantly affected by addition of 24 mg monensin/kg feed, values were 6.13 and 6.12 for control and monensin treatment respectively. Similar results also observed by Hodjatpanah, et. al., (18).

Since it was well known that feeding concentrate diets accelerated ruminal fermentation and reduced ruminal pH. It seemed that addition of monensin in a current study minimized the variation in ruminal pH may results from increasing level of concentrate. Araújo, et. al., (4) reported that feed additives such as monensin can be used to control fermentation and maintain a stable ruminal pH. Results also revealed that ruminal NH_3-N concentration was slightly increased from 6.75 to 6.97 mg/100ml due to addition of monensin. Hodjatpanah, et. al., (18) observed that ruminal NH_3-N concentration was not affected by the addition of monensin at a rate of 200 mg/day to sheep. Similar results observed by Vendramini, et. al., (40).

Results showed, that the addition of monensin led to a significant decrease ($P<0.01$) in ruminal TVFA from 14.14 to 12.10 mM/100 ml. Vendramini, et. al., (40) reported that VFA concentration was reduced ($P<0.001$) from 13.7 to 12.8 mg/100 ml as a result of inclusion of monensin at a level of 24 mg/kg DM in lamb diets. This may be attributed to absorption rate via rumen wall. Askar, et. al., (6) reported that the concentration of VFA in rumen liquor is affected by simultaneous variations in VFA absorption or rumen volume. Moreover, ruminal pH was not affected by addition of monensin in a current study, this may result in increased rate of VFA absorption across the rumen wall (10).

Effect of interaction between concentrate level and addition of monensin on fermentation parameter

Table 3 shows the effect of interaction between concentrate level and addition of monensin on fermentation characteristics. Results revealed that there were no significant differences in ruminal pH as affected by the above interaction. This result was expected since ruminal pH as previously shown was neither affected by concentrate level nor by addition of monensin. Similar results were observed by many other studies (23, 40, 43)

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Table 3- Effect of interaction between level of concentrate feeding and addition of monensin on fermentation parameter (% ± SE)

Level of conc. % of BW[1]	2.5%		3%		*P*
Addition of monensin, mg/kg conc.[2]	0	30	0	30	
Parameters of ruminal fermentation					
pH	6.72 ±0.05	7.03 ±0.06	6.91 ± 0.10	6.96 ± 0.18	NS
NH3-N, mg/100ml	6.21[b] ±0.20	6.97[a] ± 0.16	7.29[a] ± 0.11	6.97[a] ± 0.14	**
VFA, mM/100 ml	15.18[a] ± 0.38	11.44[c] ± 0.13	13.11[b] ± 0.03	12.76[b] ± 0.34	**

Means in the same row with different superscripts are significantly different
**** (P<0.01) NS= Non significant**

Regarding ruminal NH_3-N, higher (P<0.01) concentration was observed in samples withdrawn from lambs fed lower level of concentrate without addition of monensin and higher concentrate level with or without addition of monensin as compared with those offered concentrate diet at lower level without addition of monensin.

Increasing ruminal NH_3-N due to increasing level of concentrate in the diet has been reported by other studies carried out with sheep (12, 41). It was also seen that addition of monensin significantly increased concentration of this ruminal parameter. Xu, et. al., (34) explained similar increase by a reduction in the flow of microbial protein with addition of monensin without altering the intake or ruminal digestibility of nitrogen.

Results showed that addition of monensin decreased ruminal VFA concentration regardless to level of concentrate, however, the significant effect was associated with the lower level. Ruminal VFA concentration was decreased (P<0.01) by 3.74 mM/100 ml. Similarly, Merchen, et. al., (29) observed that increasing concentrate level offered to Suffolk wethers diet decreased (P<0.05) ruminal VFA concentration from 9.71 to 8.35 mM/100 ml.

Higher (P<0.01) ruminal VFA concentration was detected in samples of rumen fluid withdrawn from lambs fed concentrate diet at 2.5% of BW without addition of monensin as compared with other lambs. This result has been reported by many studies (23, 40).

In view of interaction effect between concentrate and addition of monensin, it may clearly noticed that ruminal VFA concentration was negatively affected by increasing level of concentrate in the present study. Similar case has been supposed to be attributed to the high rate of absorption of fermentation acids through the rumen wall by the effect of monensin (29).

Diurnal changes in rumen fermentation parameters

Table 4 summarizes rumen fermentation characteristics as affected by time of withdrawing rumen fluid samples from experimental animals. Statistical analysis of the data showed that higher (P<0.01) ruminal pH was detected in samples withdrawn before morning meal as compared with those in samples withdrawn 3 and 6 hours post feeding. Another aspects concerning diurnal changes was that ruminal pH was significantly (P<0.01) decreased from 7.18 in samples withdrawn before feeding to 5.80 in samples withdrawn 3 hours thereafter. Similar result was obtained by (44).

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Table 4- Effect of time of sampling on fermentation parameter (as appeared in the table ± SE)

Time of Sampling / Fermentation parameter	Before feeding	Post feeding		*P*
	0 time	3 hrs.	6 hrs.	
pH	7.18^{a} ±0.33	5.80^{c} ± 0.64	6.26^{b} ± 0.55	**
NH_3-N, mg/100ml	5.89^{b} ±0.41	8.46^{a} ± 1.24	6.23^{b} ± 0.40	**
VFA, mM/100 ml	7.35^{b} ± 0.92	15.42^{a} ± 3.46	16.61^{a} ± 2.34	**

Means in the same row with different superscripts are significantly different at
**** (P<0.01)**

Decreased pH values in the samples withdrawn 3 hours post feeding compared to the samples withdrawn before feeding is a direct result of the intake of concentrate diet. Jacques, et. al., (21) reported that the daily production of saliva was decreased with the increased level of concentrate in the diet. Decreased rumination and salivation normally are associated with high concentrate diets (8), and the consequent decrease in the buffering capacity coupled with rapid microbial degradation of soluble carbohydrates, results in a decline of ruminal pH. However, ruminal pH values were significantly increased again in samples withdrawn 6 hours post feeding. This may be due to the effect of wheat straw consumed after lambs has finished their morning meals of the concentrate, in addition to production of large amounts of saliva during chewing and rumination. Galvani, et. al., (15) stated that the ruminants secrete large amounts of saliva during chewing and rumination when eating roughage diet.

Regarding the diurnal changes of ruminal NH_3-N concentrations, the highest values (P<0.01) was detected in the rumen liquid samples withdrawn 3 hours post feeding as compared with those withdrawn before and 6 hours thereafter. As changes in ruminal pH, ruminal NH_3-N values were subjected to two clear changes. It was increased (P<0.01) from 5.89 in samples withdrawn before feeding to 8.46 mg/100ml in those withdrawn 3 hours thereafter. Then, it was decreased (P<0.01) again to 6.23 mg/100ml in samples of rumen fluids withdrawn 6 hours post feeding.

Klevesahl, et. al., (24) reported that increased concentrate level or dietary degradation typically resulted in increased ruminal NH_3-N concentration. Rasool, et. al., (34) found that the ruminal ammonia-N concentration was increased (P<0.01) at 3 hours post feeding, then it was declined at 6 hours post feeding, this may be due to the use by microorganisms or passage through the rumen wall, because during this time the ruminal was suitable for the absorption of ammonia.

Addition of monensin significantly decreased (P<0.05) ruminal NH_3-N concentrations from 20.0 to 13.4 mg/100 ml 6 hour post feeding, this was also observed due to addition of monensin to the Malpura lambs diet (9). Kim, et. al., (23) reported that addition of monensin decreased NH_3-N concentration as a result of a decrease in proteolytic and obligate amino acid fermenting bacteria because these bacteria are sensitive to ionophores.

Ruminal NH_3-N levels recorded in a current study, 5.89 to 8.46 mg/100 ml, are within the range necessary for maximum microbial growth on high-concentrate diets reported by McDonald, et. al., (28).

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Regarding the diurnal changes of ruminal VFA concentrations, results showed that higher (P<0.01) ruminal VFA concentrations were recorded in samples withdrawn from lambs at 3 and 6 hour post feeding, values were 15.42 and 16.61 mM/100 ml respectively. Similar results obtain by Dung, et. al., (13). Lower ruminal VFA concentration detected in samples withdrawn before feeding (7.35 mM/100 ml) in a current study may be attributed to absorption activity trough rumen wall.

This pattern of change in VFA concentration in ruminal liquid was expected with high pH in samples withdraw post feeding. Kim, et. al., (23) indicated the presence of a link between the high total volatile fatty acid concentration with pH of ruminal liquid. The increase in VFA content and rumen osmotic pressure can be directly related to the decrease in rumen pH (30).

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