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Effect of level of monensin on rumen fermentation characteristics in Awassi lambs

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Abstract

The current study was conducted in the Animal field of the Department of Animal Production – College of Agriculture - Al-Qasim Green University for the period from 10/10/2019 to 19/1/2020 to investigate the effect of addition of monensin at levels of 15, 30 and 45 mg/kg concentrate on growth performance of Awassi lambs. First treatment in which lambs were fed concentrate diet without addition of monensin was considered the control treatment. Sixteen male Awassi lambs were used with an average weight of 24.85 and 4-6 months of age. The lambs were randomly distributed to the experimental treatments, four lambs per each using the individual feeding in the cages. The concentrate diet was offered at a level of 2.75% of the body weight at 2 meals, morning and evening meals, while the ground wheat straw was offered at free choice. Results revealed no significant effect of level of monensin on pH values, whereas ruminal ammonia concentrations were significantly decreased ($P<0.05$) from 7.54 in the control treatment to 6.08 and 6.45 mg/100 ml due to addition of monensin at levels of 30 and 45 mg/kg. Moreover, addition of monensin at a level of 30 mg/kg concentrate diet significantly increased ($P<0.05$) ruminal concentration of total volatile fatty acids to 13.76 as compared with 10.99 mM/100 ml for control treatment. Concentrations of all rumen fermentation characteristics were significantly affected ($P<0.01$) by time of ensiling.

Key words: Monensin, lambs, fermentation, rumen

تأثير مستوى المونسين على خصائص تخمرات الكرش في الغنام العواسية

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الخلاصة:

اجريت الدراسة الحالية في الحقل الحيواني الخاص بقسم الانتاج الحيواني في كلية الزراعة-جامعة القاسم الخضراء للفترة من 2019/10/10 ولغاية 2020/1/19 للتحري عن تأثير اضافة المونسين الى علائق الحملان العواسية بمستوى 15 و 30 و 45 ملغم/كغم علف مركز على خصائص تخمرات الكرش, فيما اعتبرت المعاملة الاولى التي غذيت فيها الحملان على العلف المركز بدون اضافة المونسين معاملة المقارنة. استخدم في الدراسة 16 حمل عواسي ذكري بلغ متوسط أوزانها 24.85 كغم وتراوحت اعمارها بين 4-6 أشهر. وزعت الحملان عشوائيا على المعاملات التجريبية بواقع اربع حملان لكل معاملة باستخدام التغذية الفردية في الاقفاص. قدم العلف المركز بمستوى 2.75% من وزن الجسم الحي وبوجبتين صباحية ومسائية, فيما قدم تبن الحنطة المجروش بصورة حرة. اظهرت النتائج الدراسة عدم تأثر قيم الاس الهيدروجيني في سائل الكرش بمستوى إضافة المونسين, فيما انخفض تركيز نيتروجين الامونيا معنويا ($P<0.05$) من 7.54 في معاملة المقارنة الى 6.08 و 6.45 ملغم/100 مل عند اضافة المونسين بمستوى 30 و 45 ملغم/كغم علف مركز على التوالي. كما ادت اضافة المونسين بمستوى 30 ملغم/كغم علف مركز الى ارتفاع معنوي ($P<0.05$) في تركيز للأحماض الدهنية الطيارة الكلية الى 13.76 مقارنة مع 10.99 مليمول/100 مل لمعاملة المقارنة. وقد تأثرت جميع خصائص تخمرات الكرش معنويا ($P<0.01$) بوقت سحب نماذج سائل الكرش.

كلمات مفتاحية: مونسين, حملان, تخمرات, كرش

Introduction

The livestock industry in Iraq faces many problems and constraints, natural problems related to climate, water scarcity and human problems related to nutrition and the failure of animals to gain its requirements of nutrients. These conditions led to decrease the number of sheep by more than 45% in 2010 as compared with its number at 1961, it was about 6.663 million at 2017 (1). Due to the unique anatomical characteristics of ruminants in the presence of the rumen, where different species and strains of anaerobic microorganisms live, these animals are characterized with its ability to utilize poor quality roughages (2).

Ruminant nutritionists have made great efforts focused on improving rumen fermentation to increase production efficiency. In the last two decades, a number of active compounds that can be introduced as feed additives in ruminant diets such as monensin, the most important polycarboxylic antibiotic Ionophore compounds, have been found to achieve this goal by increasing the production of propionate while reducing the production of methane, (3).

Rutkowski and Brzezinski (4) indicated that monensin was also used as a growth promoter for ruminants and targeted specific bacterial groups in the rumen, leading to increase production efficiency. The increase in the pH of the rumen is associated with an increased level of addition of monensin to the diets offered to the ruminants (5). This is explained by the effectiveness of monensin in inhibiting lactic acid-producing bacteria such as *Streptococcus bovis* and *lactobacilli* directly (6).

Most studies indicated that introduction of monensin into ruminant diets improved the efficiency of rumen fermentation. This improvement was attributed to its positive effects on rumen fermentation, including increasing the concentration of propionate and reducing ammonia (7). Therefore, the present study aims to investigate the effect of introducing different levels of monensin on rumen fermentation characteristics.

Materials and methods

The study was conducted at the Animal fields of Animal Production Department / College of Agriculture - Al-Qasim Green University from 10/10/2019 to 1/19/2020, including a preliminary period for 30 days. Sixteen male Awassi lambs bought from local markets with an average weights of 24.85 ± 0.83 kg and 4-6 months of age were used. They were randomly distributed to experimental treatments and housed individually with four pens per each treatment. There were 4 experimental treatments based on concentrate diet offered at a level of 2.75% of body weight at 2 meals a day. Concentrate diet was offered without monensin for the first treatment (control) and with addition of monensin at level of 15, 30 or 45 mg/kg concentrate diet for the second, third and fourth treatments respectively. Table 1 shows the chemical composition of concentrate diet, its ingredients and wheat straw.

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

Ingredients	DM	DM % in						ME MJ/100 g
		Ash	OM	CP	CF	EE	NFE	
Wheat bran	93.55	5.48	95.31	14.14	13.96	4.39	62.52	1.23
Yellow corn	89.09	2.22	98.86	7.20	5.84	5.84	80.80	1.37
Barley	92.69	5.65	97.12	11.86	6.71	3.75	75.49	1.27
Soybean meal	94.31	7.87	78.62	42.79	1.47	1.47	39.35	1.18
Urea	-	-	-	287.5**	-	-	-	-
Concentrate	89.75	7.95	95.13	14.47	5.88	4.43	74.68	1.25***
Wheat straw	91.19	7.12	91.09	2.69	35.67	2.13	52.09	1.00***

* 44.41% barley, 17.85% yellow corn, 30% wheat bran and 4.90% soybean meal ** 46 × 6.25
***Level of ME in diets was estimated according to MAFF (8) equation with subsequent conversion of values from MJ/kg DM to MJ/ 100 g DM:
MAFF (MJ/ Kg DM) = 0.012 CP + 0.013 EE + 0.005 CF + 0.014 NFE.
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 (9), 70% for soybean meal (10) and 67% for wheat bran (11).
NaCl and mineral-vitamin mix manufactured by Turkish Profeed Company were added to concentrate at rate of 1% for each. Urea was added at rate of 0.536% to ensure existence of a standard ratio of 1.34 g RDN/MJ of ME (12). The estimated level of RDN in concentrate diet was about 1.67 g/100 g DM.

Withdrawal of rumen fluid sample

Rumen fluid samples were withdrawn from all lambs according to the method described by Saeed (13) using stomach tube. The samples were withdrawn within one day at 3 times, before the morning meal of concentrate diet, 3 and 6 hours after that. The samples were filtered through 4 layers of cheese cloth. The pH was measured immediately using the Mi 180 Bench Meter. Then a few drops of the 50% sulfuric acid solution were added to stop fermentations. Two fractions of acidic samples were transferred into clean pipes and kept frozen until determinations of fermentation characteristics were performed.

Determination rumen fermentation characteristics

The first part of the frozen samples were thawed and the remaining solid parts were separated using a centrifuge at 3000 rpm for 20 minutes, the supernate was then used to determine the concentration of ammonia nitrogen (NH_3-N) in the rumen liquid. 0.5 ml of the rumen liquid sample was transferred into the digestion tube of the Kjildahl apparatus and fixed in the distillation unit after addition of 0.5 g of MgO, 0.25 g of boiling stone, and 1 ml of a 25% CaCl2 solution. 10 ml of distilled water were added automatically to the tube before the start of boiling and condensation operations. The released ammonia was collected in a beaker containing 10 ml of 2% solution of boric acid with a few drops of the methyl red and green bromocresol indicator. The collected ammonia was titrated against 0.05 M of HCl solution and NH_3-N concentration was estimated according to the following equation:

$$NH_3\text{-N mg/100ml} = \frac{(\text{ml acid in titration - ml blank}) \times M \times 0.014}{\text{Sample volume, ml}} \times 100$$

To determine the concentration of total volatile fatty acids (TVFA) in the rumen liquid, the method proposed by Markham (14) was used. The second part of frozen samples were used as shown above. One ml of the rumen liquid sample was transferred into the digestion tube of the Kjildahl and 1 ml of 50% orthophosphoric acid was added. The tube was fixed in the distillation unit. Soon after operation of this unit 10 ml of distilled water were added automatically. About 50-100 ml of the condensate solution was collected in a beaker containing 3-4 drops of the methyl red and green bromomresol indicator. The collected solution was then titrated against 0.1 N of NaOH solution and the concentration of TVFA was estimated according to the following equation

$$\text{TVFA mM/100ml} = \frac{(\text{ml, base in titration - ml Blank}) \times N}{\text{Sample volume}} \times 100$$

Statistical analysis

The data were analyzed statistically according to the Completely Randomized Design (CRD) to study the effect of the level of monensin. The statistical program SAS (15) was used to analyze the data of rumen fermentation characteristics obtained in the study.

Results and Discussion

Effect of level of monensin on rumen fermentation characteristics

Table 2 shows the effect of level of monensin on rumen fermentation characteristics. Results revealed no significant effect of level of monensin on pH values. This result agrees with those obtained by many studies (16)-(19). Monensin inhibits lactic acid producing bacteria keeping pH at values within those supporting best rumen fermentation rate (20).

Table 2- Effect of level of monensin on rumen fermentation characteristics (as shown in the table ± SE).

Items	Level of monensin, mg/kg concentrate				P
	0	15	30	45	
pH	6.82 ± 0.11	6.68 ± 0.10	6.63 ± 0.07	6.79 ± 0.07	NS
NH3-N, mg/100 ml	7.54^{a} ± 0.32	6.66^{ab} ± 0.23	6.08^{b} ± 0.34	6.45^{b} ± 0.21	*
TVFA, mM/100 ml	10.99^{b} ± 0.49	12.40^{ab} ± 0.36	13.76^{a} ± 0.87	12.50^{ab} ± 0.52	*

* Means with different letters are significantly differed (P<0.05)

However, inconsistent result was reported by Rowghani, et. al., (21), addition of monensin to lambs diet at levels of 5, 11 and 22 mg/kg DM significantly (P<0.05) increased ruminal pH value from 5.83 to 5.94, 5.91 and 5.90 respectively. Similarly, Silva, et. al., (22) found a significant (P<0.05) increase in ruminal pH values from 6.07 to 6.13 due to addition of monensin to the concentrate diet offered to castrated lambs at level of 30 mg/kg.

Regarding ruminal NH_3-N concentration, results showed that it was decreased (P<0.05) from 7.54 in the control treatment to 6.08 and 6.45 mg/100 ml due to addition of monensin at levels of 30 and 45 mg/kg respectively. This result agrees with that revealed by Anassori, et. al., (17), in which introduction of monensin into the diet of Makoui lambs at a level of 33 mg/kg DM decreased (P<0.05) ruminal NH_3-N concentration after 14 days from 20.89 to 11.68 and from 21.38 to 13.33 mg/100 ml after 21 days. In other study, addition of monensin to the diet of Chall sheep decreased (P<0.05) ruminal NH_3-N concentration from 18.53 to 13.95 mg/100 ml (23).

Taghizadeh, et. al., (24) noticed a significant (P<0.05) decrease in ruminal NH_3-N concentration from 10.1 to 9.77, 9.17 and 8.63 after 2 hours and from 10.55 to10.03, 9.62 and 9.23 mg/100 ml after 4 hours of feeding due to increasing level of monensin added to the diet of Ghizel lambs from 20 to 25, 30 and 35 mg/kg. Similar results were reported by many other studies (25), (21), (22), (18).

The reduction of ruminal NH_3-N concentration observed with feeding monensin may be due to the decrease in the number of ruminal protozoa (23), (17). Many studies demonstrated that the overall and partial reduction in ruminal protozoa of sheep led to increase propionate production on expense of acetate and butyrate with a decrease in the recycled bacterial N and ruminal NH_3-N concentration (26).

Bohnert, et. al., (27) indicated that the reduction of ruminal NH_3-N concentration may associate with a decrease in the ruminal degradation of protein and peptides and in deamination of amino acids. Moreover, the reduction of ruminal NH_3-N concentration may be resulted from a decrease in the number of proteolytic bacteria or that

involved in the deamination of amino acids in the rumen (28). Joyner, et. al., (29) concluded that animal fed monensin containing diets efficiently utilize its N allowances in protein synthesis.

Inconsistence with the current study, Maas, et. al., (30) found that ruminal NH_3-N concentration was not significantly affected by the addition of monensin to the diet of castrated lambs in the Autumn (18.6 and 17.7) and Spring seasons, concentrations were 18.2 and 17.3 mg/100 ml for the addition and control treatments respectively. Similar result was obtained by Aguilera-Soto, et. al., (31), in which, ruminal NH_3-N concentration was not affected by addition of monensin to the diet of Pelibuey × Rambouillet cross bred lambs at a level of 33 mg/kg, values were 16.9 and 15.6 mg/100 ml for the addition and control treatments respectively.

Al-Shemary (19) reported different result as compared with the result of a current study. The worker fount that introduction of monensin into the concentrate diet of Awassi lambs at a level of 30 mg/kg DM increased ($P<0.01$) ruminal NH_3-N concentration from 6.21 to 6.97 mg/100 ml when lambs were fed the concentrate diet at a level of 2.5% of BW. However, no significant difference was shown when the diet was offered at 3% of BW, mean values were 6.97 and 7.29 mg/100 ml for the addition and control treatments respectively.

Results of a current study revealed also that concentration of ruminal TVFA was increased ($P<0.05$) from 10.99 to 13.76 mM/100 ml due to addition of monensin at a level of 30 mg/kg DM as compared with the control treatment. This result disagree with that obtained by Mirzaei, et. al., (23) where, addition of monensin to the diet of goat at level of 15 mg/kg had no significant effect on ruminal concentration of TVFA, values were 5.67 and 5.74 mM/100 ml for addition and control treatments respectively.

Anassori, et. al., (17) clarified that the concentration of ruminal TVFA was not significantly affected by addition of monensin to the diet of Makoui lambs at a level of 33 mg/kg DM after 14 and 21 days of the start of the study, values were 9.68 vs. 9.78 for the control treatment and 9.72 vs. 9.76 mM/100 ml for the addition treatment respectively. Al-Shemary (19) found that addition of monensin to concentrate diet of Awassi lambs at a level of 30 mg/kg decreased ($P<0.01$) ruminal concentration of TVFA from 15.18 to 11.44 mM/100 ml. Similar results were obtained by other studies (16), (25), (32), (31), (18), (24), (33).

The significant increase in ruminal concentration of TVFA due addition of monensin may be due to the improve of rumen condition and enhance activity of rumen microbes (17). In addition to the decrease of outflow rate of feed particles via fermentation area, improve digestion and processes of chewing and rumination that lead to decrease those particles and increase surface area of diets exposed to the action of microbial enzymes (34).

The differences among a current study and other studies in rumen fermentation characteristics may be due to levels of nutrients, chemical composition of organic materials, physical state of diet, concentrate to roughage ratio and method of sampling as indicated by Rowghani, et. al., (21). In that study, the ratio was 83:17%, samples were taken and data was obtained using cannulated lambs. The differences can also be attributed to the differences in study condition, such as period, formulation of diets and its characteristics, treatment or additives, level of addition and other factors (17). In addition to the level of dietary crude protein. Dung, et. al., (35) reported that ruminal NH_3-N concentration can be affected by the level of dietary CP and its ruminal degradability.

Diurnal changes in rumen fermentation characteristics

Table 3 shows the effect of sampling time on rumen fermentation characteristics in Awassi lambs. Results revealed that all studied characteristics were significantly altered. Higher ($P<0.01$) pH value of 7.36 was recorded in rumen liquid samples withdrawn before feeding in comparison with 6.37 and 6.44 recorded in samples withdrawn 3 and 6 hours post feeding. This may be due to the effect of saliva produced during chewing and rumination of roughage throughout the night

and passed to the fermentation chamber (36). Large quantities of saliva containing bicarbonate and urea may therefore flow into this chamber leading to increase ruminal pH (19).

The reduction of the ruminal pH in samples withdrawn 3 hours post feeding may be attributed to the effect of concentrate diet offered to the lambs at morning meal, in which readily fermented carbohydrates was converted by rumen microbes to lactic acid (LA) and VFA (13). Reduction of ruminal pH is mainly associated with increased LA concentration in the rumen (22).

Table 3- Effect of sampling time of rumen liquid on rumen fermentation characteristics (as shown in the table ± SE)

Time of sampling	Before feeding	Post feeding		*P*
	0	3	6	
Fermentation characteristics				
pH	7.36^{a} ± 0.05	6.37^{b} ± 0.08	6.44^{b} ± 0.08	**
NH3-N, mg/100 ml	9.52^{a} ± 0.36	7.44^{b} ± 0.21	3.09^{c} ± 0.24	**
TVFA, mM/100 ml	10.35^{b} ± 0.49	15.26^{a} ± 0.90	11.58^{b} ± 0.61	**

** Means with different letters are significantly differed (P<0.01)

Chamley (37) reported that there is a reverse relationship between intake of concentrate diet and ruminal pH. Increased acids concentration in the rumen by the time lead to reduce the pH until acids would absorbed via rumen wall (38). The linear increase in the ruminal pH at 6 hours post feeding in a current study can be explained by the fact that lambs were often moved to consume straw after it finished its concentrate meal. Taghizadeh, et. al., (24)

observed that addition of monensin to the diet of lambs stabilized ruminal pH values after 2 and 4 hours of feeding. Stability of ruminal pH values nearby neutralization limit as affected by addition of monensin have been attributed to the decrease in the number of LA producing bacteria, while those which ferment LA as a substrate are still active (5).

In consistent with a current study, Hassan and Saeed (39); Hadi (40) and Al-Shemary and Saeed (41) reported similar result. In later two studies, values of the ruminal pH in samples withdrawn 3 hours post feeding were significantly (P<0.01) decreased to 6.48 and 5.80 as compared with 7.51 and 7.18 in samples withdrawn before feeding for those studies respectively. However, inconsistently, a significant increase (P<0.01) to 6.86 and 6.26 was recorded by those workers in samples withdrawn at 6 hours post feeding. No significant difference in ruminal pH was seen in a current study between samples withdrawn 3 and 6 hours post feeding.

Regarding ruminal concentration of NH_3-N, results revealed that higher (P<0.01) value of 9.52 mg/100 ml was detected in samples withdrawn before feeding. This may be due to the effect of consuming roughage throughout the night and early morning. In this case, large quantities of saliva would excreted to moisten rough materials and facilitating swelling.

Ruminal concentration of NH_3-N was decreased (P<0.01) in samples withdrawn at 3 hours post feeding to 7.44 mg/100 ml. This decrease can be explained on basis of supplying rumen microbes with energy through morning meal of concentrate diet. This additional energy stimulates incorporation of ammonia produced from degradation of protein, non-protein nitrogen and recycled urea into microbial protein, or be utilized by rumen microbes which prefer ammonia as a N source for metabolic activity (42). Moreover, monensin plays an important role in reducing ruminal degradation of

protein and deamination of amino groups from amino acids. Taghizadeh, et. al., (24) confirmed that feeding monensin containing diets resulted in low ruminal concentration of NH_3-N due to the decrease of proteolytic bacterial activity and increased flow of protein into small intestine.

Results of a current study also showed that there was a significant ($P<0.01$) decrease in ruminal concentration of NH_3-N to 3.09 mg/100 ml in samples withdrawn 6 post feeding. This may be due to more incorporation of ammonia N in microbial protein synthesis. Al-Shemary (19) demonstrated that a decrease in ruminal concentration of NH_3-N refers to the improve of utilization of dietary N due to increase microbial protein synthesis. In addition to the inhibition effect of monensin on ruminal degradability of protein and deamination of amino groups from amino acids resulted from a decrease in the number of proteolytic bacteria and urease activity (43). Lower ruminal concentration of NH_3-N may associate with high sensitivity of ammonia producing bacteria to monensin (44), (45).

The negative effect of monensin on protozoan activity and recycling of bacterial N proposed by Jouany (46) may explain the lower ruminal concentration of NH_3-N detected in samples withdrawn 6 hours post feeding. Hussian and Saeed (47) obtained similar trend of diurnal changes in ruminal concentration of NH_3-N, there was a significant ($P<0.01$) decrease from 12.10 to 10.66 mg/100 ml in samples withdrawn 3 and 6 hours post feeding respectively. In another study, Hadi (40) found that ruminal concentration of NH_3-N was significantly ($P<0.01$) decreased from 7.57 to 6.65 mg/100 ml in samples withdrawn 3 and 6 hours post feeding respectively. The difference in the levels of ruminal concentration of NH_3-N between both studies may be due to basic diet, concentrate to roughage ratio, level of additives and how samples of rumen liquid were taken. In the first study reed silage prepared with addition of urea at 1 and 2% was used as a roughage, then degradation of dietary protein was probably occurred in addition to the rapid dissociation of urea.

Regarding diurnal changes in ruminal concentration of TVFA, higher ($P<0.01$) value was detected in samples withdrawn 3 hours post feeding, whereas lower value was detected in samples withdrawn before feeding, values were 15.26 and 10.35 mM/100 ml respectively. In those withdrawn 6 hours post feeding, a significant ($P<0.01$) decrease was detected in ruminal concentration of TVFA to 11.58 mM/100 ml.

The lower ruminal concentration of TVFA in samples withdrawn 3 hours post feeding was probably resulted from the slow degradation of structural carbohydrates presented in roughages, which its ruminal fermentation is rarely completed. While, rapid fermentation of soluble carbohydrates presented in the concentrate diet offered to lambs at morning would increase ruminal concentration of TVFA. Resende-Junior, et. al., (48) confirmed that the intake of rich carbohydrate diets may increase ruminal concentration of TVFA and decrease pH. Such case may occurred in a current study since ruminal pH was significantly ($P<0.01$) decreased 3 hours post feeding. Kim, et. al., (49) mentioned to the reverse relationship between ruminal concentration of TVFA and pH.

The decrease in ruminal concentration of TVFA in samples withdrawn 6 hours post feeding may be due to the absorption of those acids via rumen wall by simple diffusion or as negative ions. Ruminal concentration of TVFA is a result of production and absorption of these acids (50). The increase in ruminal concentration of TVFA is associated with the improve of ruminal microbial activity, especially that of cellulolytic bacteria, higher crude fiber degradation and absorption of VFA via rumen wall, it may also associate with the improve of rumen condition including stability of pH at levels that support better microbial fermentations (51).

Al-Shemary (19) reported similar trend in diurnal changes of ruminal concentration of TVFA. In his study, higher ($P<0.01$) value of 15.42 was detected in samples withdrawn 3 hours post feeding as compared with 7.35 mM/100 ml which detected in samples withdrawn before feeding. In that study,

ruminal concentration of TVFA in samples withdrawn 6 hours post feeding was 16.61 mM/100 ml. Figure 1 shows the diurnal changes in rumen fermentation characteristics.

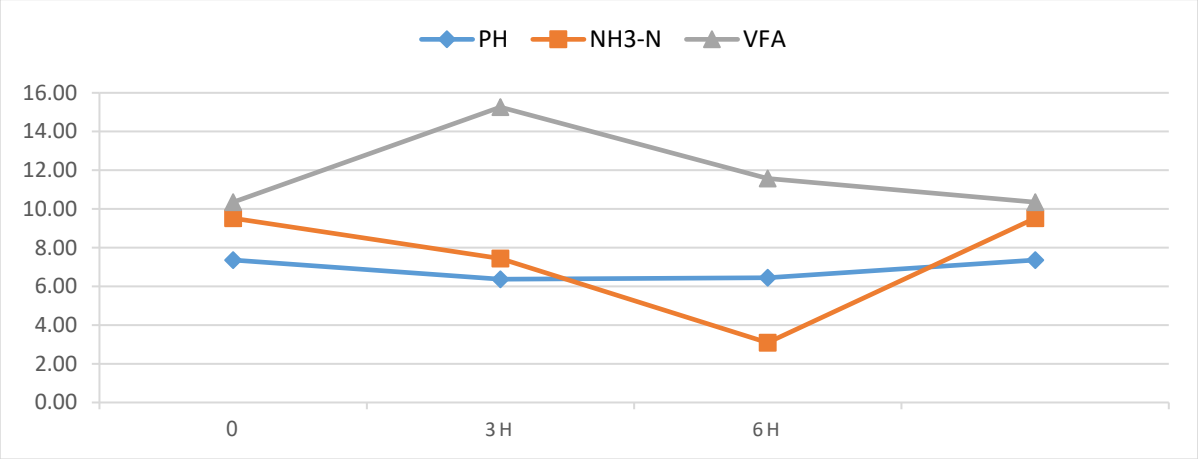

Figure 1: The diurnal changes in rumen fermentation characteristics

Conclusions

Addition of monensin to the diet of lambs enhance rumen fermentation characteristics particularly, at higher levels of 30 and 45 mg/kg concentrate diet as evidenced by low ruminal ammonia and high volatile fatty acids concentrations.

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