Effect of Feeding Different Levels of Dietary Protein and Addition of Baker's Yeast (*Saccharomyces cerevisiae*) on Rumen Fermentation Characteristics of Awassi Male Lambs

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Abstract: Twenty four individually fed Awassi male lambs were used in factorial experiment to investigate their responses to feeding concentrate diets containing three levels of dietary crude protein (CP) each was offered without or with baker's yeast, *Saccharomyces cerevisiae* (SC) at rate of 0.5% (on DM basis). Concentrates were offered at rate of 3% of live body weight with free choice of barley straw. Rumen liquids were withdrawn before feeding (0 time), 3 and 6 hrs post feeding, Results revealed that higher ruminal pH and total volatile fatty acids (TVFA) concentration ($P<0.05$) were observed in rumen liquid withdrawn from group of lambs fed the higher level of CP. Results also revealed that the molar proportion of both acetic and butyric acids were not affected by increasing level of CP. Results also revealed that addition of SC increased ruminal pH ($P<0.01$) and both TVFA concentration and molar proportion of propionic acid ($P<0.05$), while it decreased ($P<0.01$) ruminal NH_3-N concentration. In conclusion Interaction effect showed that lower ammonia N in the rumen liquid was detected in samples withdrawn from lambs fed diet containing medium level of CP and addition of SC. Higher TVFA and propionic concentrations ($P<0.05$) accompanied with lower ($P<0.05$) acetate: propionate ratio.
Keywords: Protein, yeast ,rumen characteristics, lambs

INTRODUCTION

Researchers in the field of ruminant nutrition had been interested in manipulating the ruminal ecosystem to increase production efficiency of ruminants. Manipulating rumen digestion system through the addition of direct feed microbial (DFM) and a fibrolytic enzyme to ruminant rations so as to enhance cellulose digestion and improve animal performance received the most interest in recent years (Haddad and Goussous, 2005; Hassan and Saeed,2013). Incorporation of cereal grains to ruminant diets maximizes production levels; however, this may lead to produce large quantities of lactate in the rumen (Beauchemin, et. al., 2000; Bal and Göksu, 2013). Lactate is normally utilized by specific bacteria and thus the pH is maintained above 6.0. At this pH value the cellulolytic bacteria thrive well, derive their energy requirements and produce VFA (Bowen, 2009). Incorporation of yeasts into ruminant diets is thought to help decrease ruminal lactate (Rossi, et. al, 2006), enhance utilization of NH3-N by rumen microbes and reduce acetate to propionate ratio (Lascano and Heinrichs, 2009). The objective of the current study is to investigate the effect of utilization of yeast(Saccharomyces cerevisiae) in the fattening diet of Awassi lambs on the rumen fermentation characteristics, considering performance of these farm animals as a function of changes in fermentation processes.

MATERIAL and METHOD

Twenty four Awassi male lambs weighing 26.5 kg and 4-6 months of age were used to investigate changes in rumen fermentation characteristics due to feeding three levels of dietary CP (11.5, 13.5 and 15.5%), each level was offered either alone or with the addition of baker's yeast {(*Saccharomyces cerevisiae*, SC, (0 or 0.5%)}. Concentrate diets were formulated including all these variables, and were offered to lambs at rate of 3% of live BW in addition to free choice of barley straw.

The commercial product of baker's yeast SC used as additive and it contained about 5.6×10^8 colony forming unit (CFU). Chemical analysis of diets including Dry matter (DM), organic matter (OM), crude protein (CP), ether extracts (EE) and crude fiber (CF) were determined according to AOAC (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the method of Goering and Van Soest (1970).

Rumen fluid samples were withdrawn from half of the same lambs before feeding (zero time), 3 and 6 hours post feeding using a stomach tube as described by Saeed (2008). Rumen fluid was immediately measured for pH using Orian 680 digital pH meter, USA, which was adjusted with 4 and 9 standard pH solutions. Samples were then filtered through four layers of cheesecloth to discard the sold unfermented particles. Then 10 ml subsamples were preserved by addition of 0.2 ml of 50% sulfuric acid to kill bacterial action and capture ammonia, and stored at –20 C° until subsequent analysis (Kazemi-Bonchenari, et. al., 2010).

Frozen strained rumen fluid samples were thawed at room temperature and shaken, then the contents were transferred into glass tubes and centrifuged, the supernatant was analyzed for ruminal ammonia-N by the method of steam distillation with MgO (AOAC, 1990), TVFA by the method of Markham (1942). The molar proportions of individual VFA were determined by chromatography technique using high performance liquid chromatography (HPLC) according to Zinn and Owens (1986).

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Data obtained was statistically analyzed using 3×2 factorial experiment design using completely randomized design model (CRD) procedure by (SAS, 2001). Duncan's multiple range tests was used to determine the significance of differences between treatments means (Duncan, 1955). Analysis of variance was carried out on all data. The treatments were partitioned into main effects and their interactions. Formulation and chemical composition of concentrate diets, straw are presented in Tables 1, 2 and 3 respectively.

Table 1. The formulation of experimental concentrate diets (%)

Level of CP %	11.50		13.50		15.50	
SC* %	0	0.5	0	0.5	0	0.5
Treatments no.	T_1	T_2	T_3	T_4	T_5	T_6
Ingredients %						
Barley	40	40	40	40	40	40
Wheat bran	35	35	35	35	35	35
Yellow corn	18	18	13	13	8	8
SBM	5	5	10	10	15	15
baker's yeast (SC)	0	0.5	0	0.5	0	0.5
Mineral and vitamin mixture	2	2	2	2	2	2

*Baker's yeast (SC) was incorporated as additive.

Table 2. Chemical analysis of ingredients used in formulating of concentrate diets(%).

Ingredients	Barley	Yellow corn	Soybean meal	Wheat bran
DM %	92.31	91.21	90.78	90.15
% of DM				
OM	90.32	92.60	91.23	92.00
CP	8.40	8.55	43.09	13.82
CF	6.24	3.89	5.31	9.60
EE	3.20	4.63	2.65	4.96
NFE	72.48	75.53	40.18	63.62
NDF	25.22	13.72	45.46	50.50
ADF	5.78	6.25	10.85	13.23
Cellulose	4.76	4.49	8.72	10.22
Hemicellulose	19.44	7.47	34.61	37.27
ADL	1.02	1.76	2.13	3.01

Table 3. Chemical composition of different concentrate diets and straw (% on DM basis).

Items/ diets	Concentrate diets						Barley straw
Level of CP %	11.50		13.50		15.50		
Addition of SC %	0	0.5	0	0.5	0	0.5	
DM	93.71	93.95	93.89	93.97	94.16	94.00	95.72
OM	93.47	93.72	93.97	94.49	93.62	94.18	90.19
CP	11.38	11.65	13.36	13.56	15.38	15.62	2.43
CF	7.00	6.93	7.00	6.72	6.85	6.75	40.17
EE	2.89	2.57	3.01	2.54	3.10	2.29	2.09
NFE	72.20	72.57	70.60	71.67	68.29	69.52	45.50
NDF	36.02	40.73	34.41	35.83	35.20	39.68	72.94
ADF	8.56	9.52	7.33	7.99	8.38	8.13	51.96
Cellulose	6.30	6.92	5.08	5.91	5.87	6.03	38.93
Hemicellulose	27.46	31.21	27.08	27.84	26.82	31.55	20.98
ADL	2.26	2.60	2.25	2.08	2.51	2.10	13.03
RDN	1.27	1.30	1.50	1.51	1.72	1.74	0.15
UDN	0.54	0.55	0.64	0.65	0.73	0.74	0.23
* ME	12.13	12.17	12.10	12.10	12.02	11.96	6.83

* Metabolizable energy (ME) values are estimated according to equation of Kearl (1982).

ME (MJ/kg DM) = [- 0.45 + (0.04453×% TDN)] × 4.184

TDN is estimated according to equations of Kearl (1982) as follows:

TDN for roughages (% of DM) = -17.2649+1.2120(%CP) +0.8352%NFE+2.4637% EE+0.4475 % CF

TDN for concentrate (% of DM) = 40.3227+0.5398 % CP+0.4448 % NFE+1.4218% EE–0.7007 % CF

RESULTS and DISCUSSION

Effects of level of dietary protein and addition of yeast on rumen fermentation characteristics were estimated as mean values for all sampling time during which samples of rumen liquid were withdrawn from experimental lambs, while, the diurnal changes in these parameters have been illustrated histogramatically taking into consideration that sampling time was not introduced in the statistical analysis to avoid exaggerations and complications.

Mean effect of dietary levels of protein (A) on rumen fermentation characteristics

Higher ruminal pH (P<0.05) was observed in samples of rumen liquid withdrawn from a group of lambs fed the higher level of CP. Fievez, et. al., (2001) reported that the levels of CP were significantly alter the ruminal pH. This may be attributed to higher ruminal NH_3-N may be produced due to feeding high level of CP. The increased concentration of NH_3-N with increasing CP content was observed by Gaafar, et. al., (2009). Differences in ruminal pH may also result from variations of VFA concentrations, because ruminal pH reflects the rate of fermentation of carbohydrate (Galip, 2006). Results shown in the Table 4, revealed that higher (P<0.01) ruminal NH_3-N concentration was accompanied with each increase in the level of dietary CP; Similar results were observed by Al-Malah (2007). Shamoon, et. al., (2009) and Chen, et. al., (2010) observed that NH_3-N level increased (P<0.01) with increasing CP level, the increased concentration of NH_3-N was mainly explained by a higher production of ammonia from the degradation of proteins in the rumen (Mathieu, et. al., 1996).

Table 4. Main effect of levels of dietary protein (A) on rumen fermentation characteristics

	Levels of dietary protein			Significance of effects
	Low	Medium	High	n = 72
pH	$6.74^{b} \pm 0.08$	$6.76^{b} \pm 0.08$	$6.98^{a} \pm 0.07$	*
NH_3-N mg/ 100 ml	$16.79^{c} \pm 0.68$	$20.20^{b} \pm 0.74$	$23.59^{a} \pm 0.74$	**
TVFA mmol/l	$111.25^{b} \pm 2.43$	$112.87^{b} \pm 2.38$	$119.47^{a} \pm 2.11$	*
Acetic acid (%) C_2	64.59 ± 0.36	63.17 ± 0.25	63.52 ± 0.20	NS
Propionic acid (%) C_3	$20.56^{c} \pm 0.27$	$22.54^{a} \pm 0.23$	$21.09^{b} \pm 0.16$	*
Butyric acid (%) C_4	11.90 ± 0.19	11.20 ± 0.09	12.06 ± 0.23	NS
C_2:C_3	$3.15^{a} \pm 0.05$	$2.80^{c} \pm 0.03$	$3.01^{b} \pm 0.02$	*

Means having different letters at the same row are significantly different.
* (P<0.05) ** (P<0.01) NS= non significant

Ruminal TVFA concentration increased significantly (P<0.05) with high level of dietary CP, similar result was obtained by Christensen, et. al., (1993) though, insignificant differences were detected with low and medium levels; this agrees with other findings demonstrating no effect of increasing level of CP on TVFA (Chen, et. al., 2010). Since ruminal concentration of VFA is the net result of their production and absorption and the rate of absorption increases as ruminal pH decreases (Dijkstra, et. al., 1993), the higher concentration of TVFA observed with high level of CP in the current study may be attributed to lower absorption through the rumen epithelium as evidenced by higher ruminal pH recorded with this level. Higher ruminal NH_3-N and TFVA accompanied with high level of CP may refer to ruminal condition leading to inefficient synchronization between rate of N and energy release. Results obtained also revealed that the molar proportion of both acetic and butyric acids was not significantly affected by increasing level of CP, Similarly; Christensen, et. al., (1993) indicated that the dietary CP level did not alter the molar proportions of ruminal fluid of acetate, propionate, butyrate or the molar ratio of acetate to propionate. In the current study, propionic acid was significantly increased (P<0.05) due to increasing level of CP; Higher concentration of this acid was observed with medium level; This may be attributed to the better ruminal condition leading to more efficient microbial activity (Chumpawadee, et. al., 2006). Improvement of ruminal propionic acid due to increasing dietary CP especially with the medium level resulted in decreased (P<0.05) acetic: propionic ratio; Therefore, a similar trend of changes in propionic acid concentration and acetic: propionic acid ratio was observed. Diurnal changes in rumen fermentation parameters as affected by increasing levels of dietary CP are presented in Figures 1, 2, 3, 4, 5, 6 and 7.

Main effect of addition of yeast on rumen fermentation characteristics

Addition of yeast resulted in higher (P<0.01) ruminal pH. A similar result was observed by many other studies (Yang, et. al., 2004 and Gaafar, et. al., 2009). This buffering effect could be resulted from a decrease in the ruminal lactate concentrations. Nisbet and Martin (1991) demonstrated that SC stimulates the uptake of lactate by *Selenomonas ruminantium*. This bacterium is one of the most important consumers of lactic acid that have been shown to be *in vitro* stimulated by yeast in an incubation of mixed rumen fluid (Newbold, et. al., 1998).

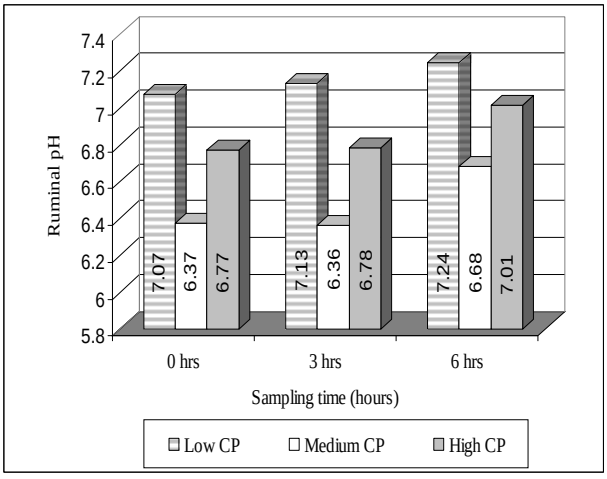

Figure 1. Diurnal pattern of ruminal pH

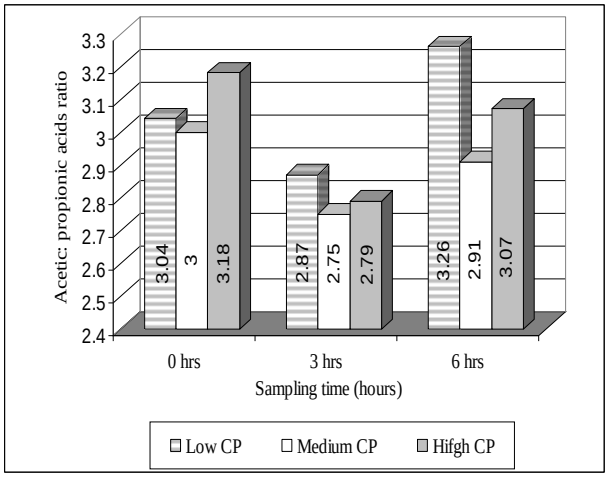

Figure 7. Diurnal pattern of ruminal acetic acid to propionic acid ratio

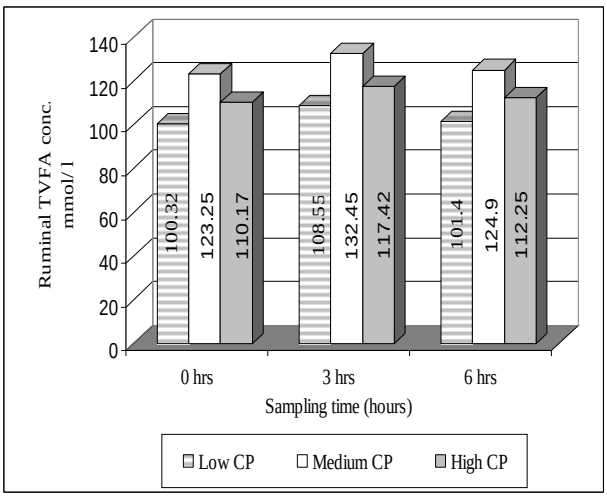

Figure 3. Diurnal pattern of ruminal TVFA acid molar proportion (%)

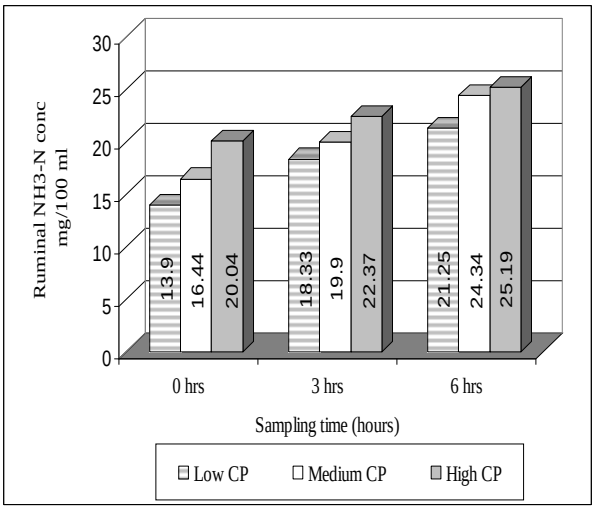

Figure 2. Diurnal pattern of ruminal NH_3-N concentration (mg/100 ml)

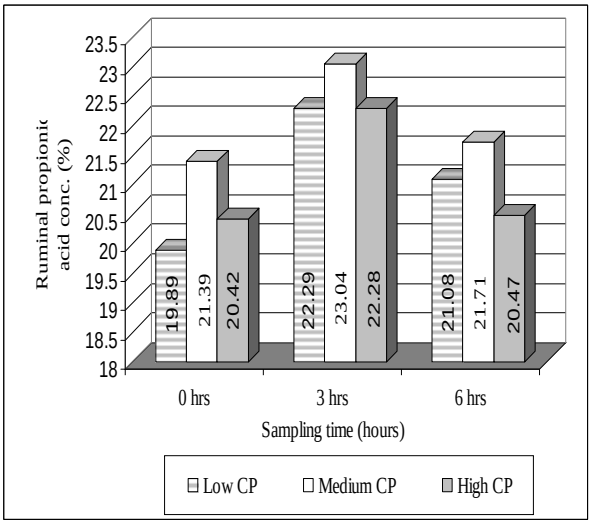

Figure 5. Diurnal pattern of ruminal butyric acid molar proportion (%)

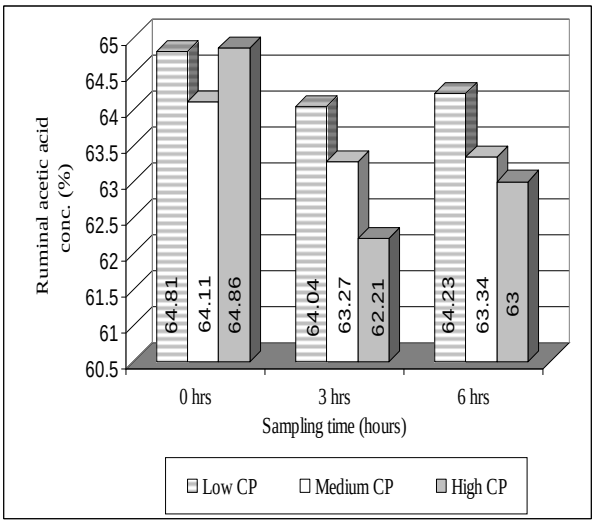

Figure 4. Diurnal pattern of ruminal acetic concentration (mmol/l)

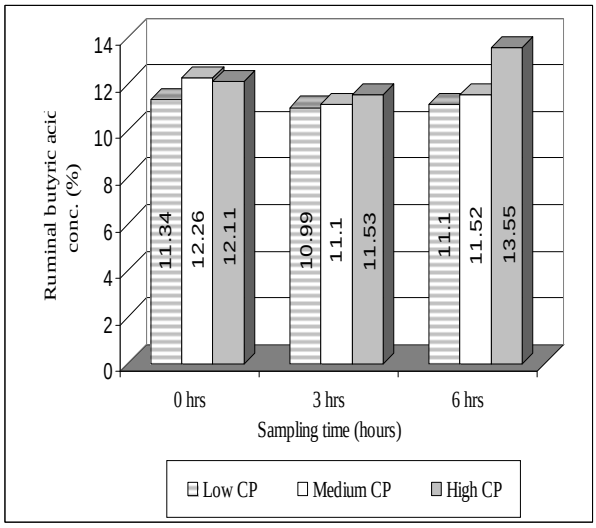

Figure 6. Diurnal pattern of ruminal butyric acid molar proportion (%)

Yeast is also able to compete with *Streptococcus bovis*, the main lactic acid producer in the rumen, for soluble sugars uptake (Chaucheyras, et al., 1996). Mathieu, et al. (1996) suggested that protozoa are involved in the effect of SC on the increase of rumen pH. Doreau and Jouany (1998) and Chevaux and Fabre (2007) observed that in individual animals, yeast reduced daily fluctuations in pH values and also decreased differences existing between them. This resulted in a higher stability of rumen environment during the day and may explain the highly significant increase detected in the current study. Our results(Table 5) also revealed that ruminal NH_3-N concentration was significantly decreased ($P<0.01$) due to addition of SC, similar finding was reported by many studies (Khadem, et. al., 2007 and Lascano and Heinrichs, 2009).

Table 5- Main effect of addition of SC (C) on rumen fermentation characteristics

	Addition of SC		Significance of effects
	Without	With	n = 72
pH	$6.66^{b} \pm 0.06$	$6.99^{a} \pm 0.06$	**
NH_3-N mg/ 100 ml	$22.04^{a} \pm 0.71$	$18.34^{b} \pm 0.65$	**
TVFA mmol/l	$111.58^{b} \pm 1.83$	$117.48^{a} \pm 1.97$	*
Acetic acid (%)	64.32 ± 0.25	63.20 ± 0.20	NS
Propionic acid (%)	$20.82^{b} \pm 0.18$	$21.97^{a} \pm 0.23$	*
Butyric acid (%)	11.50 ± 0.16	11.94 ± 0.15	NS
C_2;C_3	$3.09^{a} \pm 0.04$	$2.88^{b} \pm 0.03$	*

Means having different letters at the same row are significantly different.
* ($P<0.05$) ** ($P<0.01$) NS= non significant.

This decrease may be resulted from increased incorporation of ammonia into microbial protein (Chaucheyras and Fonty, 2001), and stimulation of microbial activity (Lascano and Heinrichs, 2009), or it may be a direct effect of yeast on reducing the degree of CP degradation (Eweedah, et. al., 2005). Our results concerning ruminal pH and NH_3-N disagree with those obtained by Yoon and Stern, (1996) who reported no significant effect of addition of yeast on ammonia-N concentration or pH of the rumen fluid. This disagreement may be attributed to the differences in the level of addition and/or different strains of SC used. Newbold, et. al., (1995) reported that some strains of yeast is effective whereas others are not.

Addition of SC led to a significant increase ($P<0.05$) in ruminal TVFA and propionic acid, whereas, both acetic and butyric acids concentrations were not affected. The beneficial effect of SC addition on TVFA concentrations was previously observed (El-Ghani, 2004 and Galip, 2006). This increase may be attributed to the enhancement of microbial activities (Erasmus, et. al., 1992); Ruminal microbial growth was stimulated through making use of specific soluble growth factors such as organic acids, B vitamins and AA provided by added yeast (Nisbet and Martin, 1991). The positive effect of addition of SC in the current study agrees with findings obtained by many workers (Newbold, et. al., 1996; Guedes, et. al., 2008). The increase in ruminal propionic acid may be represented the end products of lactate metabolized by lactate utilizing bacteria that stimulated by addition of yeast (Kung and Hession, 1995). Propionogenesis is often improved by yeast (Erasmus, et. al., 1992), but sometimes acetogenesis is stimulated (Chaucheyras, et. al., 1995); consequently, the increase in ruminal propionate in the current study, seemed to be occurred at the expense of acetate. Similar result was shown by Plata, et. al., (1994). The reduction in ruminal acetate, though, insignificant in our results, is confirmed by in vitro study carried out by Lynch and Martin (2002). As expected, higher ruminal propionate accompanied with lower acetate concentrations due to addition of SC, resulted in a significantly ($P<0.05$) decrease in C_2:C_3 ratio. Similarly, Williams, et. al., (1991); Erasmus, et. al., (1992) and Lynch and Martin (2002) reported that acetate: propionate ratio was decreased due to supplementation with yeast. Diurnal changes in rumen fermentation parameters as affected by addition of yeast were presented in Figures 8, 9, 10, 11, 12, 13 and 14.

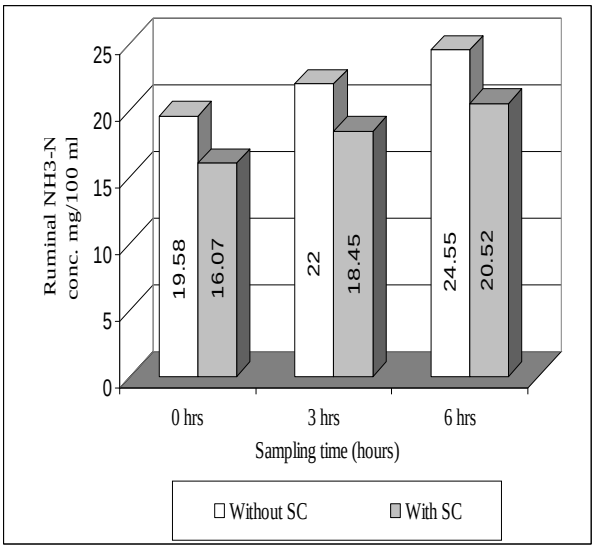

Figure 8. Diurnal pattern of ruminal pH

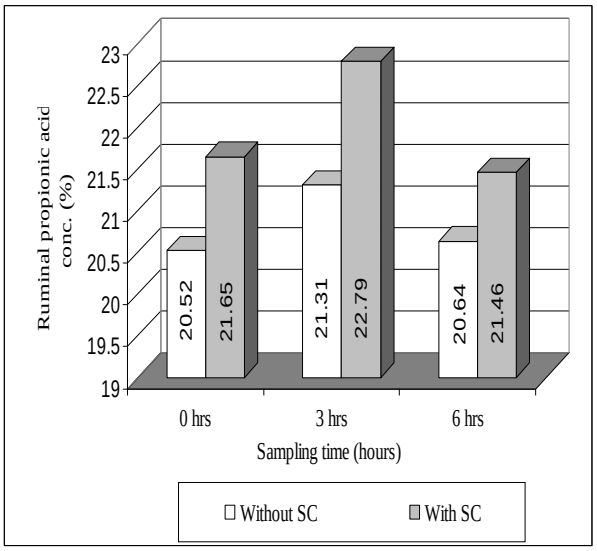

Figure 13. Diurnal pattern of ruminal acetic: propionic acid concentration (%)

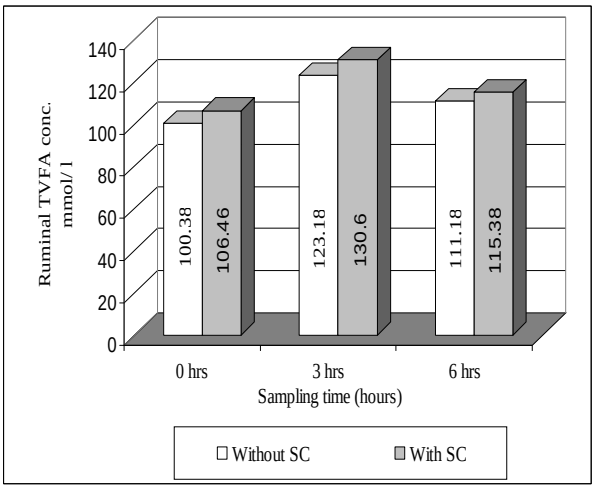

Figure 9. Diurnal pattern of ruminal TVFA concentration (mmol/l)

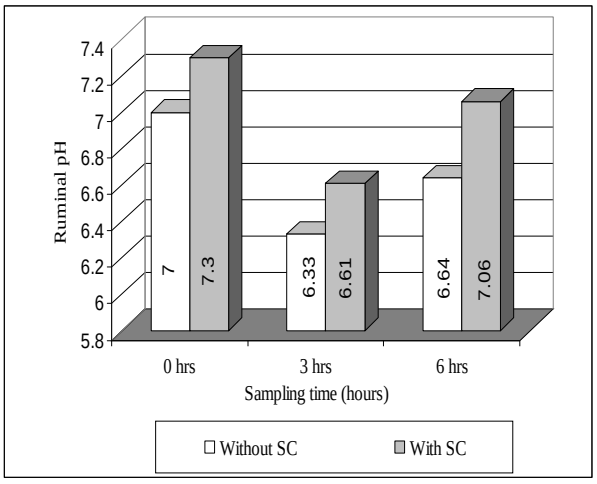

Figure 10. Diurnal pattern of ruminal NH_3-N concentration (mg/100 ml)

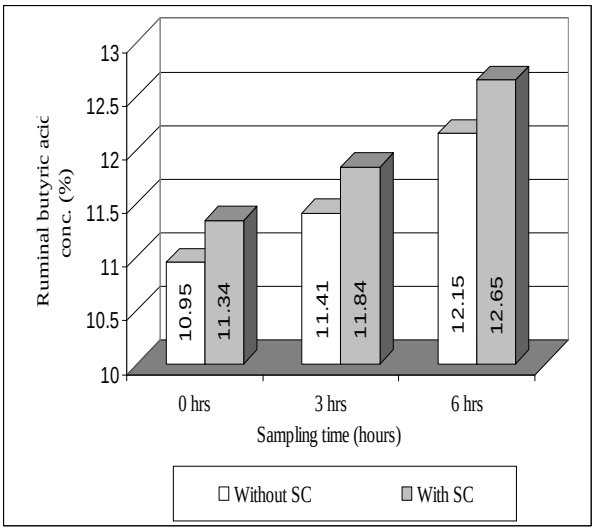

Figure 11. Diurnal pattern of ruminal propionic acid molar proportion (%)

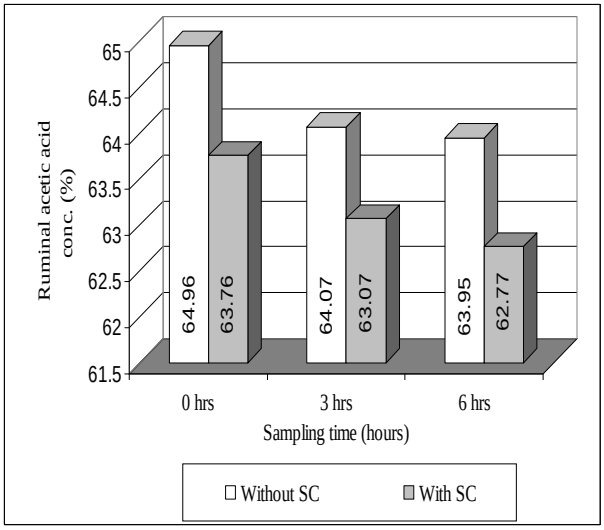

Figure 12. Diurnal pattern of ruminal acetic acid molar proportion (%)

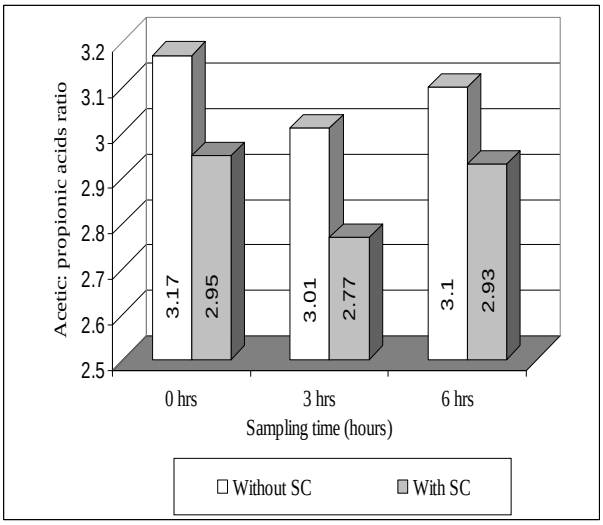

Figure 14. Diurnal pattern of ruminal butyric acid molar proportion (%)

Mean effect of the interactions between levels of dietary protein and addition of SC (A×C) on rumen fermentation characteristics

Ruminal pH was significantly (P<0.05) affected by this interaction (Table 6). Higher value was accompanied with a high level of dietary CP. Similar finding was observed by Fievez, et. al., (2001). As mentioned earlier, this may be attributed to higher ammonia produced (Gaafar, et. al., 2009). Ruminal NH_3-N concentration was significantly (P<0.01) affected by the above interaction, where, higher values occurred with increasing level of dietary CP. Similar results were demonstrated by many studies (Shamoon, et. al., 2009 and Chen, et. al., 2010).

The increased concentrations of NH_3-N may be attributed to the ruminal degradation of CP (Mathieu, et. al., 1998). However, addition of SC decreased these values within each level of CP; similar results were observed by many investigations (Khadem, et. al., 2007 and Lascano and Heinrichs, 2009).

This decrease may refer to increased incorporation of ammonia into microbial protein (Chaucheyras and Fonty, 2001), due to stimulation of microbial activity (Lascano and Heinrichs, 2009) by soluble growth factors such as organic acids, B vitamins and amino acids, that it was believed to be supplied by SC (Waldrip and Martin, 1993). Scavenging excess oxygen from the rumen (Newbold, et. al., 1996) was another assumption. Ruminal TVFA was significantly (P<0.05) affected by the studied interaction. Results revealed that within each level of CP, addition of SC led to increase this parameter; Improvement of ruminal TVFA concentration due to addition of yeast was mentioned by works of (El-Ghani, 2004 and Galip, 2006). This improvement can be explained through the stimulation effect of SC addition on microbial activities (Erasmus, et. al., 1992). Results also showed that propionic acid and subsequently, acetic: propionic acid ratio were affected significantly (P<0.05) by this interaction, where, higher ruminal propionic acid concentration were observed due to addition of SC; Similarly, Kung and Hession, (1995) observed increased ruminal propionic acid concentration due to stimulating fermentation processes of ruminal transient lactic acid by SC. Higher concentration of propionic acid in addition to lower acetic: propionic acid ratio was detected in rumen liquid withdrawn from a group of lambs fed medium level of CP with added SC; Authors indicated that the effect of addition of SC to ruminant's diets may be propionogenic (Erasmus, et. al., 1992). Results of the current study revealed that this process was occurred as evidenced by increase propionic and decrease acetic acid concentrations and this may refer to better rumen condition which may reflect better performance.

Table 6. Mean effect of the interactions between levels of dietary protein and addition of yeast (A×C) on rumen fermentation characteristics

	Interactions						P
	A_1C_1	A_1C_2	A_2C_1	A_2C_2	A_3C_1	A_3C_2	n = 72
pH	$6.57^{c} \pm 0.11$	$6.90^{ab} \pm 0.10$	$6.59^{c} \pm 0.12$	$6.92^{ab} \pm 0.10$	$6.81^{bc} \pm 0.08$	$7.14^{a} \pm 0.11$	*
NH_3-N mg/ 100 ml	$18.31^{c} \pm 0.87$	$15.27^{d} \pm 0.88$	$22.48^{b} \pm 0.93$	$17.92^{c} \pm 0.71$	$25.35^{a} \pm 0.96$	$21.84^{b} \pm 0.90$	**
TVFA mmol/l	$105.90^{c} \pm 2.91$	$116.60^{ab} \pm 3.31$	$115.60^{ab} \pm 3.11$	$123.35^{a} \pm 3.36$	$113.25^{bc} \pm 3.06$	$112.50^{bc} \pm 3.04$	*
Acetic acid (%)	65.49 ± 0.49	63.69 ± 0.39	63.67 ± 0.37	62.67 ± 0.28	63.81 ± 0.23	63.24 ± 0.31	NS
Propionic acid (%)	$19.93^{d} \pm 0.34$	$21.20^{bc} \pm 0.34$	$21.68^{b} \pm 0.16$	$23.40^{a} \pm 0.27$	$20.87^{c} \pm 0.23$	$21.31^{bc} \pm 0.23$	*
Butyric acid (%)	11.66 ± 0.32	12.14 ± 0.18	11.04 ± 0.13	11.37 ± 0.11	11.80 ± 0.31	12.32 ± 0.34	NS
C_2:C_3	$3.29^{a} \pm 0.08$	$3.01^{b} \pm 0.05$	$2.93^{b} \pm 0.03$	$2.67^{c} \pm 0.03$	$3.05^{b} \pm 0.03$	$2.96^{b} \pm 0.03$	*

Means having different letters at the same row are significantly different.* (P<0.05) ** (P<0.01) NS= non significant.
A_1, A_2 and A_3 represent low, medium and high level of CP respectively, $C_1$and C_2 represent 0 and 0.5% of SC level respectively

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