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EFFECT OF PROTECTED METHIONINE SUPPLEMENTATION ON PRODUCTIVE PERFORMANCE OF GROWING BUFFALO CALVES

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SUMMARY

Fifteen male buffalo calves with an average of initial live body weight ranged between 250 and 267 kg were used to study the effect of rumen-protected methionine (RPM) supplementation on growth performance, nutrients digestibility and some ruminal parameters of growing male buffalo calves. Calves were randomly assigned into three nutritional groups (each of five animals) to receive one of the following experimental rations: the first group served as a control (T_1) which received the basal ration (without methionine supplementation); (T_2) received the basal ration (T_1) and was supplemented with 15 g RPM /h /d; and (T_3) received the basal ration (T_1) plus 25 g RPM /h /d. Results obtained indicated that different experimental rations had in general almost similar chemical composition. Adding RPM to basal ration of buffalo calves, led to significant improvement ($P<0.05$) in experimental rations digestibility and nutritive values (TDN and DCP). Results of ruminal parameters indicated insignificant improvement ($P>0.05$) in different ruminal parameters, due to RPM supplement to the basal ration, while time of sampling indicated significant differences ($P<0.05$) for pH, NH_3-N and TVFA's values at different times. Data obtained pointed out also to positive insignificant impact of RPM supplementation on accelerating calves daily gain and FCR. Efficiencyof feed utilization (FCR) was highest in T_3 followed by T_2in compare with T_1, however, differences were statistically non-significant ($P<0.05$).

Keywords: *protected methionine, productive performance, buffalo calves.*

INTRODUCTION

Protein is an important limiting nutrient in ruminants fed low quality forages. It becomes essential when animal reaches its optimum growth or peak production. This is because nutrients requirements of ruminant animals differ according to the physiological state, like; growth, pregnancy and lactation (Ali *et al.* 2009). All Ruminants need two types of digestible protein. The first is a bacterial protein produced by the rumen microbes (microorganisms) to produce microbial protein. The second is the true protein in the diet that escapes degradation in the rumen (rumen undegradable protein RUP or bypass protein), which is digested in the small intestine and used by the animals themselves. Rumen degradable protein is used to synthesize microbial protein, which is a valuable metabolizable protein source for the animals; metabolizable protein consists of by-pass protein and microbial protein. Even though the microbial protein alone is likely sufficient enough to meet the needs of cattle at maintenance. On contrarily, growing cattles and lactating cows are in need to bypass protein, in addition to microbial protein to meet their metabolizable protein (Kamalak *et al.* 2005).

Methionine (Met) is most likely to be the first or second most limiting amino acids in most diets of beef cattle (Merchen and Titgemeyer, 1992), mainly when the amino acids profile available at the duodenum closely approximates the profile of rumen synthesized microbial protein. Ruminally protected methionine (RPM) by-passes the ruminal degradation, because of the coating process, and enters the small intestine where it can be directly absorbed. Methionine is also, involved in many pathways including the synthesis of phospholipids, carnitine, creatine and polyamines (Berthiaume *et al.* 2006). In addition, methionine is the source of the methyl donor S-adenosyl methionine, the metabolite that provides methyl groups in a variety of reactions including the de novo synthesis of choline from phosphatidylethanolamine. Thus, balancing rations with ruminally protected methionine supplementation to improve the profile of essential amino acids in metabolizable protein is fundamental to allowing the feeding of lower levels of dietary crude protein and rumen undegradable protein (RUP), optimize growth and retained nitrogen in ruminant animals (Loerch and Oke, 1989). Ruminally protected methionine improves gain and feed conversion efficiency in growing cattle (Wright and Loerch, 1988). Studies conducted on Holstein steers demonstrated that supplementation of ruminally protected methionine improved average daily gain (ADG) and protein efficiency (Alonso *et al.* 2008). Therefore, the objective of the present study was to evaluate the effect of rumen-protected methionine (RPM) supplementation to the basal ration on ration nutrients digestibility, rations nutritive values and growth performance of male buffalo calves.

MATERIALS AND METHODES

The present study was carried out at the experimental farm station belongs to the Faculty of Agriculture, Al-Azhar University, Mostorod, Qalyubia Governorate, Egypt, through the period from December 2015 to September 2016.Animals in different groups were fed a basal ration (14.3 % CP), according to NRC recommendation (NRC 1981).

Fifteen male buffalo calves with an initial live body weight ranged between 250-267 kg were randomly assigned into three nutritional groups (each of five animals / group) to receive one of the following experimental rations; the first group served as a control (T_1); the second group (T_2) received the basal ration (T_1) which was supplemented with 15g RPM / head/ day; (T_3) received the basal ration (T_1) plus 25 g RPM/head/day. Rations were offered *ad lib* and residuals were daily weighed. A digestibility trail was conducted according to Abou-Akkada and El-Shazly (1958). Samples of rations offered and residuals if any, were daily weighed during the collection period for further chemical analysis. Samples of ruminal fluids were collected before feeding (0 hrs), 3 and 6 hrs post feeding using rubber stomach tube. Rumen liquor was used to determine immediately pH values (using Orion Research Digital pH meter, model 201), TVFA's concentration according to Warner (1964), while NH_3-N concentration mg/100ml was determined according to Conway (1962). Samples of feeds and faeces, were analyzed for dry matter (DM),crude protein (CP), crude fiber (CF), ether extract (EE), ash contents according to A.O.A.C. (1990).

Statistical analysis

Data were statistically analyzed according to SAS version 9.1 (SAS 2002). The significance among treatment means was tested by Duncan's Multiple Range Test (Duncan, 1955). The statistical model used was:

$$Y_{ij} = \mu + T_i + M_j + E_{ij}$$

WhereY_{ij}=the observation of the parameter measured, μ=theoverallmeans, T_i=fixed effect of dietary treatment, M_j=fixedeffect of sampling time and E_{ij} is the random error term.

RESULTS AND DISCUSSION

Chemical composition of the experimental rations (DM basis %).

The chemical composition of the experimental rations on dry matter basis is presented in Table (1). As shown in, similar DM and OM contents were observed with different CFM samples, *i.e.* 89.00 % DM, 91.53-91.6% OM, 14.30-14.38 % CP, 8.74 % CF, 4.2 % EE, 64.21 - 64.36 % NFE and 8.40 – 8.47 ash for T_1, T_2 and T_3 , respectively. It was noticeable that, inclusion of (RPM) in rations (T_2 and T_3) led to increase slightly, rations crude protein content.

Digestibility coefficients and nutritive values of the experimental rations (DM basis %)

Results of dry matter intake, digestibility coefficients of different nutrients and nutritive values of different experimental rations are presented in Table (2).

- **Dry matter intake (kg/h/d)**

Dry matter intake (DMI) for different tested rations did not differ significantly ($P<0.05$) among different groups. However, T_1 (Control) recorded the higher dry matter intake *i.e.* (9.15 kg/h/d) followed by T_2 (8.69 kg/h/d). While (T_3) recorded the lowest intake value (8.54 kg/h/d).

Similar results were obtained by Alonso *et al.* (2008) whoreported a reduction in DM intake due to supplementation of 10 g (RPM) to rations of growing heifers (*Bos indicus*) raised in humid tropics. These results agreed well with those obtained by Wang *et al.* (2010), using (RPM); Gajera *et al.* (2013), using

(RPM) with by-pass lysine and fat in growing Jaffrabadi heifers and Singh *et al.* (2015), who pointedout to insignificant effect due to feeding (RPM) with different levels of soybean meal, neither on DMI nor on rations nutrients digestibility values.

Table (1): Proximate chemical analysis of the experimental rations

Ration	Chemical composition of CFM %						
	DM	OM	CP	CF	EE	Ash	NFE
T_1	89.00	91.60	14.30	8.74	4.2	8.40	64.36
T_2	89.00	91.56	14.34	8.74	4.2	8.44	64.28
T_3	89.00	91.53	14.38	8.74	4.2	8.47	64.21
	Chemical composition of Roughage %						
Wheat straw	93.12	89.20	1.79	39.71	0.45	10.80	47.25

Table (2): Feed intake, digestibility coefficients and nutritive values of the experimental rations.

Item	Experimental rations		
	T_1	T_2	T_3
Dry matter intake kg/h/d			
Total DM intake (kg)/h/d	9.15±0.97	8.69±0.71	8.54±1.37
Digestibility Coefficients %			
DM	60.06^{b}±0.25	61.68^{a}±0.15	61.97^{a}±0.14
OM	59.04^{b}±0.20	60.54^{ab}±0.29	61.25^{a}±0.78
CP	58.21^{c}±0.18	59.93^{b}±0.21	62.70^{a}±0.63
EE	72.25^{b}±0.22	73.07^{b}±0.20	75.20^{a}±0.28
CF	59.79±0.13	61.00±0.10	61.35±0.46
NFE	58.21^{b}±0.39	59.83^{a}±0.24	61.12^{a}±0.48
Nutritive values %			
TDN	56.85^{b}±0.24	58.29^{ab}±0.20	59.66^{a}±0.51
DCP	6.82^{b}±0.03	7.09^{ab}±0.05	7.52^{a}±0.17
C/P ratio	8.34^{a}±0.03	8.23^{a}±0.04	7.94^{b}±0.11

a , b, and c ; means with different superscripts in the same row are significantly different from each other (P<0.05).

• Digestibility Coefficients %

Results obtained in Table (2) showed digestibility coefficients for different experimental rations. As shown, DM digestibility was improved (P<0.05) from 60.06 to as high as 61.97% for (T_3); OM was also improved (P<0.05) from 59.04 to 61.25 % (T_3). Crude protein (CP) digestibility increased (P<0.05) from 58.21 to 62.70 for (T_3); Ether extract (EE) increased from 72.25to as high as 75.20% for (T_3) and NFE from 58.21 % to 61.12 %, respectively, due to (RPM) supplementation to T_2 and T_3 rations. However, insignificant higher (P<0.05) crude fiber (CF) digestibility was detected for T_3 (61.35 %) in compare with T_1 and T_2. It was noticeable that, the improvement in nutrients digestibility of the two supplemented rations (T_2 and T_3) was in parallel to the ascending level of (RPM) supplementation, *i.e.* from 15 to 25 g to the basal ration (Control), respectively. The improvements in nutrients digestibility due to RPM (Table 2), agreed with those obtained by El-Ganiny *et al.* (2007) who reported that nutrients digestibility was increased with (RPM). Results of the present study are in a good agreement with those obtained by Gaafar *et al.* (2011), who found that digestibility of DM, OM, CP and ether extract were significantly (P<0.05) higher in the methionine group compared with the control group. On the contrarily, Sun *et al.*, (2009) reported that increasing the level of methionine supplementation neither improved (P<0.05) nutrients intake nor digestibilities. Similarly, Obeidat *et al*, (2008) reported that supplementing the diet of growing Awassi lambs with rumen-protected methionine at 0, 7 or 14 g/ d / head neither affect nutrients intake nor nutrients digestibilities.

- **Nutritive values %**

Nutritive values of different experimental rations (Table 2) indicated, in general higher (P<0.05) values for different supplemented rations with (RPM) in compare with that of the control one. The highest TDN value was observed with diet contained 25 g methionine /head/d (T_3), (59.66 %) followed by T_2 which contained 15 g methionine /head/d (58.29 %), and without significant differences between them. While the lowest TDN value was recorded by the control group (56.85 %). Similar improvement (P<0.05) in DCP was also detected due to RPM supplementation, *i.e.* 7.09 and 7.52 % for T2 and T3, respectively, in compare with the basal ration (nil RPM supplement). And as a general observation, the improvement in TDN and DCP contents of the two supplemented rations was coincide with the corresponding improvement occurred in nutrients digestibility values, and tended to be more obvious with the parallel increase in RPM supplement, i.e. from 15 to 25 g/h/d. The improvements in nutritive values due to RPM (Table 2) agreed with those obtained by El-Ganiny *et al.* (2007) and Gaafar *et al.* (2011) whoreported that supplementation of methionine to the rations, resulted in significant increases (P<0.05) in TDN and DCP values for rumen protected methionine groups compared with that of the control group. Calorie to protein ratio (Table 2) indicated, in general proper percentages (7.94 to 8.43), which covers the daily requirements of growing buffalo calves DCP and TDN demands at such age and live body weight. The improvement in digestibility coefficients could be attributed to the fact that methionine may play an indirect role in the stimulation of anaerobic fermentation of organic matter, which improves the efficiency of nutrients utilization and had a direct role in the improvement of abomasal digestion.

Ruminal parameters of calves fed rations containing rumen protected methionine

1. Rumen pH

Results of ruminal pH, total volatile fatty acids (TVFA's) values and ammonia nitrogen (NH_3-N) concentration are illustrated in Table {3).

Table (3): Effect of protected methionine supplementation on some ruminal parameters of buffalo calves at different times of sampling.

Item	Sampling time	Experimental rations			Overall mean for time
		T_1	T_2	T_3	
pH	0	7.13	7.12	7.13	$7.12^a \pm 0.03$
	3	6.59	6.64	6.58	$6.60^b \pm 0.07$
	6	6.71	6.77	6.88	$6.79^b \pm 0.08$
	Overall mean	6.81 ± 011	6.84 ± 0.09	6.86 ± 0.10	
NH_3–N (mg/100ml)	0	17.75	17.79	17.83	$17.79^c \pm 0.11$
	3	26.22	26.20	26.20	$26.21^a \pm 0.13$
	6	19.36	20.44	20.49	$20.10^b \pm 0.30$
	Overall mean	21.11 ± 1.31	21.47 ± 1.25	21.51 ± 1.25	
VFA's (meq/100 ml)	0	8.58	8.61	8.60	$8.60^c \pm 0.10$
	3	9.88	9.91	9.90	$9.90^a \pm 0.11$
	6	9.40	9.48	9.55	$9.48^b \pm 0.11$
	Overall mean	9.29 ± 0.22	9.33 ± 0.21	9.35 ± 0.22	

a, b and c small letters; means with different superscripts in the same column indicated significant differences at (P< 0.05).

Ruminal pH is one of the most direct and important parameter which reflect animals rumen fermentation status. It is also an important factor for altering rumen microbial protein synthesis.The average value for pH of rumen liquor,herein the present study 6.81, 6.84 and 6.86 respectively (Table 3), for different animals groups, indicating insignificant differences (P<0.05). The obtained values were within the normal ranges (6.81- 6.86) reported by Hungate (1966), who indicated that cellulytic bacteria need a rumen pH of about 6.2 and 7.0 in order to multiply rapidly and colonize the epidermal surfaces of plant fragments, within 5 minute. This observation in the present study is in agreement with that of Noftsger *et al.,* (2005) who observed that rumen pH was not different across diets. In similar and previous experiments, increasing the CP content of the diet did not lead to affect rumen pH and consistently enhanced the concentration of NH_3-N in the rumen (Cunningham *et al.*, 1996). However, rumen protected choline without or with rumen protected methionine supplementation were found to reduce insignificantly rumen pH by about 1.6 and 1.6 %, respectively when compared with limited Met and RP-Met treated cows, respectively (Soltan*et al.*, 2012).

On the contrarily, the averages values for pH concentration at 0, 3 and 6 h after feeding, in the ruminal liquor of calves fed with the experimental rations (Table 3) revealed that time of measuring had significant affect ($P<0.05$) on rumen pH value. Ruminal pH values tended to decrease by prolongation of time post-feeding, reaching to lower value (6.60) at 3 h post-feeding, then it tended to increase after 6 h post feeding. According to Hiltner and Dehority (1983), within 6 h after feeding, the ruminal pH of all treatments was greater than 6.3, which pH was identified as critical for maintaining ruminal fiber digestion. Numerous investigations indicated similar results like that, with protected methionine supplementation and ruminal fluid pH, which coincide with the present results (Table 3). Abdelmawla (1997) showed that pH values decreased 2 h after feeding. The decrease in pH values 3 h post feeding may be a result to ruminal TVFA's production. However, matching TVFA's production values of different experimental groups with the corresponding pH values (Table 3) indicated insignificant differences in both criteria due to (RPM) supplement to the basal ration. This result might lead to conclude that; supplementation of RPM did not affect TVFA's production. Hence, pH values of different animals groups did not significantly altered. According to the results of Boraei, (2010) ruminal pH values fell within the normal range 3 h post feeding for optimum cellulytic bacteria activity. On the other side, Afzalzadeh *et al.* (2010) reported that increasing rumen degradable protein from 9.8 to 11.8 % resulted in non-significant linear decreases in ruminal pH ($P<0.01$).

2. Rumen ammonia-N concentration

Rumen ammonia-N concentration is normally affected by rumen wall absorption and chyme emptying velocity (Allison, 1980), which is a reflection of rumen microbial decomposition of nitrogenous substances and utilization of ammonia. Results of NH_3-N (Table 3) showed that, there were insignificant differences in NH_3-N values among the two supplemented rations compared with that of the control one. Ruminal NH_3-N values, as shown, recorded insignificantly higher ($p<0.05$) values for T_3 (21.51 mg/100 ml) and T_2 (21.47 mg/100 ml) than the control group (21.11 mg/100 ml). These results were in agreement with those reported by Noftsger *et al.* (2005) who found that ammonia in the rumen was not different (approximately 11.8 mg/dL) and was well above the 5 mg / dL minimum suggested for maximal bacterial crude protein synthesis (Satter and Slyter, 1974). Sampling time (Table 3) indicated higher ($P<0.05$) NH_3-N release at 3 hrs (26.21 mg / 100 ml) and 6 hrs (20.10 mg / 100 ml) after feeding in comparison with those recorded at zero time (17.79 mg/100 ml). Matching NH_3-N concentration at the corresponding pH values at different times of measuring, indicated higher ($P<0.05$) NH_3-N production at pH 6.60 (3 hrs post feeding) lowered ($P<0.05$) to (20.10 mg/ 100 ml) at pH 6.79 (6 hrs after feeding), but only 17.79 mg/ 100 ml at pH 7.12 (before feeding).This result might lead to suggest that, at pH value ranged between (6.60 - 6.79) and corresponding to 3 and 6 hrs post feeding, seems to be the proper pH media for ruminal bacteria activity; a result which was obviously detected in a significant increase ($P<0.05$) in ruminal NH3-N production, (Table 3). While Yang *et al.*, (1986) indicated that, the concentrations of ruminal ammonia 2 to 4 h after feeding were similar for cows fed both diets (heat treatment of soybean meal without or with added ruminally protected methionine). This finding, they added might suggest that the protein in heat treatment of soybean meal (HSBM) plus methionine diet was degraded at a slower rate by the ruminal microorganisms when methionine was supplemented, or it might suggest improved microbial and peripheral nitrogen utilization. They referred the lower ruminal pH ($P<0.05$) in methionine supplemented cows to the lower ($P>0.05$) rumen ammonia concentrations together with higher TVFA's concentrations.

3. Total Volatile fatty acids

Ruminal microorganisms convert dietary protein and carbohydrate to volatile fatty acids (VFA's), microbial protein and gases. The volatile fatty acids and microbial protein can be absorbed and utilized by the host animal, whereas the gases are lost to the environment. The end products of ruminal fermentation contain useful compounds, for example TVFA's, microbial protein and water soluble vitamins, besides useless compounds, such as CH_4 and CO_2, and even compounds harmful to the host animal, like ammonia and nitrate (Owens and Goetsch, 1993). Volatile fatty acids (VFA's) are important products of microbial fermentation in the rumen and their composition and content in the rumen might be influenced by, rumen environment, microbial population and dietary composition. Least squares means for TVFA's concentrations presented in Table (3) pointed out to insignificant effect ($P<0.05$) of RPM supplementation on concentrations of total volatile fatty acids production. Both of the protected methionine supplementation (T_2 and T_3) recorded slightly higher, but insignificant values in compare with the control ration. Values obtained were 9.33, 9.35 and 9.29 meq/100 ml, respectively.

Results of TVFA's (Table 3) indicated in general, relative improvement in TVFA's production for calves fed rations containing protected methionine, although values were not significant, the matter which might be referred to the increase in DM, OM, CP and NFE digestibility than those of control ration. Russell and Dombrowski (1980) reported that ruminal TVFA's production was closely related to ruminal pH, which can be considered as an important regulator of microbial yield. Furthermore, depression of both ruminal pH value with the rise in TVFA's and NH_3-N concentration may be attributed to the proper functioning of rumen microorganisms, which utilize ammonia-N to synthesize microbial protein as reported by Breves and Schroder (1991). Therefore, it can be concluded that the supplementation of protected methionine supplementation had to somehow a stimulatory effect on rumen fermentation. However, rumen fermentation was found to be obviously enhanced after feeding rations rich in degradable CP, rather than undegradable ones. Values of TVFA's in the present study were similar also to that reported by Yang *et al.,* (1986) who indicated that total TVFA's concentrations in ruminal fluid were similar for both treatments (heat treatment of soybean meal without or with added RPM). Data presented in (Table 3) concerning time of sampling indicated significant differences ($P<0.05$) in TVFA's concentration of the rumen liquor. As shown, different treatments tended to have lower TVFA's concentration at 0 time (before feeding) which tended to increase 3 hrs post feeding which declined later at 6 hrs post feeding. Data of TVFA's indicated in general, significant TVFA's concentration at different sampling time *i.e.* 8.60, 9.90 and 9.48 at 0, 3 and 6 hrs post feeding, respectively.

Effect of rumen protected methionine supplementation to basal ration on calves performance

- **Animal growth performance**

According to numerous authors, growing cattle fed diets low in ruminally undegraded protein (RUP) would benefit from the supplementation with limiting amino acids (Din *et al.* 2009), and supplementation with methionine, the first limiting AA, in diets with deficient bypass protein was found to improve nitrogen retention (Greenwood and Titgemeyer, 2000).

Results presented in Table (4) indicated insignificant differences among different experimental groups in final live body weight (Kg), total body weight gain (Kg), daily gain (g) and growth rate in percentages. However, the final live body weight, total body weight gain (kg) and average daily body weight gain (gm) /h/ day during the experiment period were highest in 25 g RPM supplemented group (T_3) followed by 15 g RPM supplemented group (T_2), which differed but insignificantly ($P<0.05$) from that of control group (T_1).

Results obtained in the present study are similar to those observed by Veira *et al.* (1991) and Van Amburgh *et al.,* (1993) in steers fed rumen protected methionine plus lysine in their ration. Similar findings were also reported by Hussein and Berger (1995), Wiese *et al.,* (2003) and Noftsger and St-Pierre (2003) who found that body weights and live body weight gain did not differ by treatment.

Growth rate in percentage (Table 4), as the value of final live body weight / group relative to the corresponding initial one, favored T_3 (25 g RPM group) as the most gaining and performing group *i.e.*104.13 %, followed by T_2 (15 g RPM group) 95.75 % and T_1 (control group) 81.14 %. In contrast, other studies reported an obvious improvement ($P<0.05$) in growth rate, dry matter intake and feed efficiency (Tripp *et al.,* 1998 and Wright and Loerch, 1988) when growing cattle were supplemented with RPM.

The inconsistency shown in different results, when animals were supplemented with RPM could be attributed partially to diets composition, kind of protected Amino acid used, degree of protection and the growth stage of animal production. Microbial proteins reaching the duodenum and dietary proteins escaping ruminal degradation provide the amino acids required by ruminants and determine the order of the limiting amino acids (Merchen and Titgemeyer, 1992). Therefore, dietary composition could influence the ruminant's response to RPM. Moreover, the level of dietary CP would influence the response of growing ruminants to RPM.

Results of feed intake for different experimental groups (Table 4) indicated also insignificant intake values in term of total dry matter intake (kg) /h /d, however, T_1 (control group) recorded higher insignificant intake value (9.15 kg /h / day and 7 % higher than that of the T_3 (25 g RPM) group intake.

Results herein agree with those reported by Leonardi *et al.* (2003) who pointed out to insignificant differences in dry matter intake among groups, due to methionine supplementation to basal ration.

Table (4): Effect of protected methionine supplementation on growth performance and feed conversion ratio of growing buffalo calves.

Item	Experimental rations			± SE
	T_1	T_2	T_3	
Initial body wt.(kg)	267.33	250.00	256.67	37.92
Final body wt.(kg)	483.00	471.00	510.33	40.06
Total body gain (kg)	215.67	221.00	253.67	20.12
Daily gain (g)	0.80	0.82	0.94	0.07
Growth rate (%)*	81.14	95.75	104.13	17.79
Daily feed intake ± SE(kg/h/d)				
Roughage (kg) /h/d	1.88	1.74	1.66	0.41
CFM(kg)/h/d	7.27	6.94	6.88	0.47
Total DM feed intake(kg)/h/d	9.15	8.68	8.54	0.87
TDN (kg)/h/d	5.20	5.06	5.08	0.51
DCP (kg) /h/d	0.62	0.61	0.64	0.07
Feed conversion (Kg intake/kg gain)				
DM(kg)/kg gain	11.41	11.17	9.16	0.80
TDN (kg)/ kg gain	6.49	6.51	5.45	0.65
DCP (kg) /kg gain	0.78	0.79	0.69	0.09

**: % growth rate = Total body gain/ Initial LBW x100.*
Feed conversion = kg of feed consumed per kg of live weight gain.

According to the present results and that of another authors, supplementing the basal ration with RPM, unless it did not lead to increase animal daily dry matter intake, but it tended to improve insignificantly animals feed utilization, more efficiently. As shown in Table (4), both of T_2 and T_3 which showed lower insignificant DMI, exhibited higher insignificant feed conversion ratios (FC) in different feeding terms, *i.e.* DMI, TDNI and DCPI /kg gain. Similar results were obtained by Chen *et al.* (2011) who studied the effect of feeding different sources of rumen-protected methionine on milk production and N-utilization in lactating dairy cows and they reported that, treatments neither affect significantly DMI nor animal live body weight gain. Feed intake for different experimental groups in term of TDN, indicated insignificant differences among groups. Control group (T_1) recorded higher TDN intake (5.20 kg /h /day), however, differences among different groups were not significant. This result might be referred to either the higher DMI of ration (T_1) or to the higher TDN value of the two supplemented rations, *i.e.* T_2 and T_3 (58.29 & 59.66) TDN, respectively (Table 2).

As for DCP intake (kg /h /day), no significant differences were detected among groups; T_3indicated higher DCP intake kg/ h / day (0.64 kg / h / day). This result might be also referred to the higher DCP content of the two supplemented rations, *i.e.* 7.09 and7.52 % DCP, respectively, due to its higher digestibility values (Table 2). The results of the present study are in agreement with those reported by earlier researchers, *i.e.* Singh *et al.* (2015) who mentioned that digestible nutrients intake *i.e.* (TDN), (DCP), digestible dry matter (DDM) and digestible organic matter (DOM) intake were also not affected (P>0.05) by supplementation of rumen protected methionine.

Effect of rumen protected methionine (RPM) supplementation on feed conversion

Feed conversion ratio was calculated as kgs dry matter required per kg of body weight gain. Feed conversion for different experimental groups as a good indicator to animal performance indicated that the efficiency of feed utilization was highest in T_3 followed by T_2 and T_1, although differences were not statistically significant (P<0.05). Figures of feed conversion ratios values were 11.41, 11.17 and 9.16 Kg DMI/kg gain, respectively. These findings are in agreement with similar results, that evaluated the effect of rumen protected Methionine in Jaffrabadi heifers (Odedra 2013) and Wiese *et al.* (2003) in growing calves on fortification of rumen protected methionine.However, results reported by Singh *et al.* (2015) pointed out to significant reduction (P<0.001) by 25 and 27% in T_1 and T_2, respectively indicating better efficiency of converting feed into body mass.

In many another studies, it was reported an improvement in growth rate and feed efficiency (Wright and Loerch 1988) when growing cattle were supplemented with RPM. The inconsistency in the results when animals were supplemented with RPM could be attributed partially to diet composition, kind of amino acids used, degree of amino acids protection and the growth stage of animal production.

On the light of the present results (Table 4), it could be concluded that, supplementing the basal ration with RPM at 15 and 25 g/h/d, led to improve significantly basal ration digestibility, nutritive values and insignificantly ruminal fermentation measurements and animals feed utilization in different feeding terms. Moreover, the improvement in growing male buffalo calves daily weight gain and feed conversion ratio was found to be insignificantly more pronounced with the linear increase in (RPM) level from 15 to 25 g /head/ day.

CONCLUSION

It could be concluded that supplementing the basal ration with varying levels of protected methionine supplementation led to variable insignificant positive effect in improving buffalo calves performance, led to improve ($P<0.05$) rations nutrients digestibility and nutritive values and tended to have insignificant influences on improving animals ruminal activity, buffalo calves gain and feed conversion ratio. However, the insignificant improvement in feed conversion ratios recorded in the present study was suggested to be mainly related to the lower ($P<0.05$) feed intake / kg gain, and the higher ($P<0.05$) nutritive value of the two supplemented rations rather than to real increase in calves daily gain. It was suggested to experience the utilization of protected methionine with either rations of low nutritional values or to be used in a mixture with other protected proteins or amino acids to achieve more pronounced and obvious results.

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تأثير إضافة الميثيونين المحمي على الأداء الإنتاجي لعجول الجاموس النامية

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أجريت هذه الدراسة بمحطة البحوث الزراعية التابعة لقسم الإنتاج الحيواني بكلية الزراعة جامعة الأزهر – مسطرد – محافظة القليوبية خلال الفترة من ديسمبر 2015م إلى سبتمبر 2016م. الهدف من هذه الدراسة هو دراسة تأثير استخدام الميثيونين المحمي كإضافات غذائية لعلائق عجول الجاموس على تحسين القيم الغذائية للعلائق وتحسين كفاءة الأداء للحيوانات المغذاه عليها مقارنة بالعجول التي غذيت على العليقة الضابطة (الكنترول).غذيت الحيوانات أثناء فترة الدراسة على عليقة اساسية (14.3 % بروتين) طبقاً للتوصيات القياسية. استخدم في هذه الدراسة 15 عجل جاموسي بمتوسط وزن بداية يتراوح ما بين 250 - 267 كجم ، تم تقسيم الحيوانات عشوائياً إلى 3 مجاميع غذائية (5 حيوانات للمجموعة) – وحيث تمت تغذيتها على العلائق التالية: المعاملة الأولى (T_1): وفيها غذيت الحيوانات على العليقة الأساسية للمزرعة بدون أية إضافات ، المعاملة الثانية (T_2) وفيها غذيت الحيوانات على العليقة الأساسية بالإضافة إلى دعمها غذائيا بالميثيونين المحمي بمعدل 15 جرام/ للرأس/يوم ، المعاملة الثالثة (T_3) وفيها غذيت الحيوانات على العليقة الأساسية وتم دعمها غذائيا بالميثيونين المحمي وبمعدل 25 جرام/للرأس/يوم.تمت تغذية الحيوانات تغذية حرة *adlib* مع حساب البواقي المتخلفة يومياً.تم وزن الحيوانات في بداية فترة التجربة، ثم تكرر الوزن بعد ذلك بمعدل مره كل أسبوعين وحتى نهاية فترة الدراسة بغرض التقدير الواقعي للاحتياجات الغذائية اليومية للحيوانات وحساب الكفاءة التحويلية للغذاء. تم إجراء تجربة هضم أثناء فترة الدراسة على الحيوانات المعاملة وبمعدل 3 حيوانات لكل معاملة غذائية لتقدير القيمة الهضمية للعلائق التجريبية، كما تم سحب عينات من سائل الكرش لتقدير بعض خصائص و صفات سائل الكرش (الأس الهيدروجيني، تركيز الأمونيا بالملجرام /١٠٠سم٣ سائل كرش وكذا مجموع الأحماض الدهنية الطيارة). أظهرت نتائج التحليل الغذائي للعلائق المستخدمة في هذه الدراسة تشابهاً في عناصر التحليل الكيميائي لمكونات الغذاء كنسب مئوية ، وإن أظهرت النتائج المتحصل عليها من تجارب الهضم وجود اختلافات معنوية عند مستوى (5 ٪) في معاملات هضم معظم مكونات العلائق المختبرة نتيجة لتدعيم هذه العلائق بالميثيونين المحمي فيما عدا معامل هضم الألياف (CF). أظهرت العلائق المدعمة بالميثيونين المحمي تحسنا معنويا (5 ٪) في القيم الغذائية للعلائق متمثلا في محتواها من المركبات المهضومة الكلية (TDN) والبروتين الخام المهضوم (DCP) بالمقارنة بعليقة الكنترول (بدون إضافات). أما فيما يختص بمقاييس التخمر لسائل الكرش ، فلقد أظهرت النتائج المتحصل عليها عدم وجود فروق معنوية بين المعاملات عند مستوى (5 ٪) نتيجة لتدعيم عليقة الكنترول بالميثيونين المحمي على أي من مقاييس التخمر التي تم دراستها والتي اشتملت على قياس درجة حموضة الكرش ، مستوى الأمونيا وكمية الأحماض الدهنية الطيارة الناتجة ، وإن أظهرت النتائج وجود اختلافات معنوية عند مستوى (5 ٪) باختلاف زمن أخذ العينات. وبالنسبة لتأثير تدعيم العليقة القياسية بالميثيونين على كفاءة عملية النمو وأداء عجول الجاموس فقد أظهرت النتائج وجود تحسن وإن كان غير معنوي في كفاءة النمو والإنتاج لهذه الحيوانات، وإن أظهرت المعاملات المدعومة بالميثيونين المحمي انخفاضا غير معنوي في معدلات استهلاك الغذاء اليومي المأكول على أساس المادة الجافة أو المركبات المهضومة الكلية.أوضحت النتائج أيضا وجود تحسن، وإن كان غير معنوي في كفاءة التحويل الغذائي لعجول الجاموس المدعومة بالميثيونين المحمي في علائقها وتزيد درجة التحسين وإن كان غير معنوي بزيادة مستوى الميثيونين من 15 إلى 25 جم /رأس / يوم (عليقة 2 ، 3 على الترتيب).

بناءً على النتائج المتحصل عليها فإنه يمكن القول بأن: استخدام الميثيونين المحمي بمستويين (15 ، 25 جم / رأس / يوم) كإضافات غذائية في علائق عجول الجاموس النامية قد أدى إلى تحسين معاملات الهضم لمركبات العليقة وتحسين قيمها الغذائية مقدرة على صورة مركبات مهضومة كلية وبروتين خام مهضوم ، كما ظهر تحسن وإن لم يكن معنويا في كفاءة التخمرات داخل الكرش وتحسن في معدلات النمو اليومية وفي كفاءة عملية التحويل الغذائي للحيوانات المعاملة.