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EFFECT OF SUPPLEMENTING RATION WITH THIAMIN AND/OR SODIUM BICARBONATE ON RUMINAL FERMENTATION, DIGESTIBILITY AND SERUM PARAMETERS OF RAMS.

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SUMMARY

Four rams were used in 4x4 Latin square design with 21-d. periods. The four treatments were: G1) control diet. G2) control diet supplemented with 34 mg thiamin, G3) control diet supplemented with 34 mg thiamin plus 16 g sodium bicarbonate ($NaHCO_3$) and G4) control diet supplemented with 16 g $NaHCO_3$ g/d/h. The control diet consisted of 50% green forage (Darawa) and 50% concentrate feed mixture (on DM basis). The main results indicated that differences in ruminal pH and NH_3-N among all groups were not significant ($p>0.05$), whereas, total volatile fatty acids (TVFA's) concentrations were significantly higher ($p<0.05$) for group G3 comparing with group G1. Concerning results of digestibility, all nutrients digestibilities were not statistically different ($P>0.05$), except protein digestibility of G3, which significantly ($P<0.05$) decreased compared with the control. Blood serum GOT and GPT showed non significant differences among all groups in there activities. It could be concluded that thiamin alone or with $NaHCO_3$ supplementation to rams rations improved ruminal TVFA's, without deleterious effects on animal health.

***Keywords:** thiamin, $NaHCO_3$, rams, rumen, digestibility, blood serum*

INTRODUCTION

Although most of the vitamins in B-complex group are synthesized in satisfactory amounts in the rumen for normal functions, there is a new approach to add some of these vitamins to ruminants' diets. For example: Folic acid , vitamin B_{12} , choline, riboflavin and biotin were added to diets of ruminants (Girard 1998 and Santschi *et al.*, 2005).

Thiamin (Vitamin B_1) is an another one added to ruminants' ration under certain conditions. Thiamin is a complex of nitrogenous base containing a pyrimidine ring linked to a thiazole ring by a methylene bridge. Yeast is the richest plant source of thiamin, at the same time, egg yolk and liver are the richest animal source. Thiamin acts as a coenzyme for certain enzymes which are necessary for energy metabolism (McDowell 2000) .

Ruminants could be come deficient in thiamin in certain cases such as: 1) inadequate net microbial synthesis of thiamin, 2) impaired absorption or utilization of thiamin, 3) presence of thiamin ant metabolites (thiaminase), 4) increased metabolic demand for thiamin and 5) increased rate of thiamin excretion (Grigat and Mathison , 1982).

When diet of steers, fed only concentrates, was supplemented by 1.9 mg thiamin /kg diet, daily gain was significantly highly (1.4 kg/d) than control (0.92 kg /d). Moreover thiamin supplementation tended to increase milk and milk components production when dietary concentration of neutral and acid detergent fiber were lower and non fiber carbohydrate was higher than recommended (Grigat and Mathison, 1982) and (Shaver and Bal , 2000).

With regard to $NaHCO_3$, it was found that addition of $NaHCO_3$ to diets of ruminants increased ruminal pH. (Kohn and Dunlof ,1998), increased ruminal efficiency of microbial N captur (Larsen,1981) and increased ruminal TVFA's (Schmidely,2005). Also cows fed maize silage (MS) without $NaHCO_3$ addition consumed 1.24 kg/d less DM ($p<0.02$) than cows fed buffer (Wenping *et al.*. 2005). Moreover no difference ($p<0.05$) in neutral detergent fiber (NDF) intake was observed with the addition of sodium bicarbonate to diets cows. (Kawas *et al.*, 2007)

The present work was undertaken to evaluate the effect of thiamin and/or $NaHCO_3$ supplementation on nutrients digestibility, rumen liquor and blood serum parameters of rams .

MATERIALS AND METHODS

This study was carried out at the Agricultural Experimental Farm in Faculty of Agriculture, Cairo University, Giza, in cooperation with Dairy Science Department, National Research Centre, Dokki, Giza, Egypt, during summer season in 2006.

Animals and feeding:

Four rams weighed an averaged (58 kg and adapted in four metabolic cages were used in a 4x4 Latin square trial. Rams were assigned randomly to experimental diets. Each experimental period extended for a 18 days preliminary period followed by 3 days collection period. At the end of each period . experimental diets were switched according to preplanned assignments. Dietary treatments were (G1) control: (G2) control + 34 mg thiamin/h/day: (G3) control +(34 mg thiamin and 16 gm sodium bicarbonate ($NaHCO_3$))/h /day; (G4) control +16 gm $NaHCO_3$/h /day. The control diet consisted of Darawa and concentrate feed mixture (CFM) (as 1:1 on DM basis). Thiamin was (imported from China) obtained from ADWIA

Company, Egypt . The chemical composition of feed ingredients and control diet are presented in Table (1). Nutrients requirements were calculated according to NRC (2001).

Feces Sampling:

Feces were taken during the collection periods from each animal. Sub sample (10%) of total collected feces was sprayed with 10% sulfuric acid , and then dried at 70°C for 24 hour. Concentrate feed mixture, Darawa and feces samples were analyzed for the gross composition (A.O.A.C., 1995) to determine the digestibility coefficients of nutrients, total digestible nutrients (TDN) and digestible crude protein (DCP).

Rumen liquor sampling

At the end of each period, rumen liquor samples were withdrawn by stomach tube before morning feeding and at 3.00 hrs and 6.00hrs post morning feeding. Samples were directly strained through three layer of gauze . They were tested for pH value using digital pH meter, NH_3-N concentration was determined according to A.O.A.C. (1995) and TVFA'S was determined according to Warner (1964).

Blood sampling

Blood samples were taken at the end of each digestibility trial before morning feeding and at 4.00 hrs after morning feeding from jugular vein from all animals. Collected blood samples were centrifuged at 4000 r.p.m. for 20 min. and the supernatant was stored in glass vials at – 20° C till analysis. Serum total protein was determined according to Gornal et al. (1949), albumin (Doumas, *et al.*, 1971), urea (Fawcett and Scott 1960), transaminses (GOT and GPT)activities (Reitman and Frankel 1957), glucose (Trinder 1969), and total cholesterol (Allain et al., 1974). Globulin, and albumin to globulin ratio (A/G) were calculated.

Statistical analysis:

Analysis of variance for digestibility was conducted according to Snedecor and Cochran, (1982) using Latin Square design, where the model was:

$$Y_{ijk} = U + T_i + P_j + A_k + E_{ijk}$$

However, data collected from rumen liquor and blood serum were statistically analyzed according to the following model:

$$Y_{ijk} = \mu + T_i + P_j + (T*P)_{ij} + E_{ijk}$$

where, Y: stands for every observation of the K^{th} animal in the J^{th} period given I^{th} treatment, T: treatment effect, P: periods effect, A: animal effect and E: the experimental error. Differences were tested by Tukey test.

RESULTS AND DISCUSSION

Ruminal fermentation

Data of Table (2) clearly showed that diets supplemented with thiamin or thiamin plus $NaHCO_3$ resulted in minute decreases ($p>0.05$) in pH values comparing with unsupplemented diet.

$NaHCO_3$ supplementation as 16g /h/d (G4) insignificantly (p>0.05) increased the pH value up to 6.70 comparing with that of G1 (6.64). This result may reflect that thiamin is not a buffer such as $NaHCO_3$.

With regard to ruminal TVFA's, data of Table (2) illustrated that the addition of 34 mg thiamin, 34 mg thiamin plus 16 g $NaHCO_3$ or 16g $NaHCO_3$ /h /d to diets of rams tended to increase the TVFA's concentration by 20.40, 31.14 and 11.14%, respectively, compared with the control diet. These increases were only statistically significant (p<0.05) for groups 2 and 3. The finding of (G3) may be possibly related to the associated effect of thiamin and $NaHCO_3$, thiamin may affect the buffering capacity of $NaHCO_3$ and the later may affect the thiamin action as a coenzyme for carbohydrates metabolized by ruminal microorganisms. The similarity of the roughage to concentrate ratio (50:50)among the experimental groups may emphasize this possibility.

However, Kawas *et al.* (2007) suggested that ruminal TVFA's concentration of lambs, fed all-concentrate with/or without yeast culture and sodium bicarbonate, was not differed among treatments. The conflicting trend between the present and Kawas *et al.* (2007) studies might be related to R:C ratio difference and yeast is considered as B-complex not thiamin only.

Data of Table (2), clearly indicated that supplementation of thiamin with or without $NaHCO_3$ had no remarkable significant (p>0.05) effect on the concentration of NH_3-N in the rumen. The obtained results of NH_3-N concentration in both G1and G4 were in line with those reported by Kennelly *et al.* (1999) who studied the effect of carbohydrates level (50%) and buffer ($NaHCO_3$) on rumen fermentation in cows.

Digestibility and nutritive values

Thiamin or $NaHCO_3$ supplementation did not affect (p>0.05) the whole tract digestibility of DM, OM, CF, EE and NFE (Table 3). Also, diets supplemented with 34mg thiamin (G2)or 16g $NaHCO_3$/h/d (G4) had no significant (p>0.05) differences on CP digestiblity comparing with control group (G1) (Table3).

The present data had the same trend obtained for diets of cows supplemented by buffer ($NaHCO_3$) or B-complex or yeast or buffer plus yeast (Kennelly *et al.* 1999, Kawas *et al.* 2007 and Majee *et al.* 2003). However, rams fed diets supplemented with both thiamin and $NaHCO_3$ had significant lower digestibility of CP (67.53%) (p<0.05) comparing with unsupplemented group (G1) (74.62%) (Table 3). This finding might reflect that thiamin plus $NaHCO_3$ supplementation affected unwell on ruminal protein degradation and or abomasums protein digestion negatively.

With regard to nutritive values expressed as TDN and DCP, it could be reported that no significant differences in TDN (p>0.05) were dictated among the experimental groups. However, diets

Table (1) : The chemical composition of feed ingredients and control diet .

Item	DM%	DM basis %					
		OM	CF	CP	EE	NFE	Ash
CFM*	90.25	93.50	11.10	14.92	4.33	63.15	6.50
Darawa	22.2	89.64	30.00	12.60	2.70	44.34	10.36
Control diet**	56.23	91.57	20.55	13.76	3.51	53.75	8.43

*CFM: Concentrate feed mixture consisted of 49% maize, 24% wheat bran ,24% undecorticated cotton seed meal, 2% limestone and 1% sodium chloride.
**Calculated.

Table (2): Effect of diets supplemented with thiamin and sodium bicarbonate ($NaHCO_3$) on some ruminal parameters of rams .

Item	Experimental Groups*					Overall mean of sampling time		
	G1	G2	G3	G4	SE	0hr	3hr	6hr
pH	6.64	6.53	6.55	6.70	$0.15^{n.s}$	6.89	6.50	6.44
TVFA's (meq/dl)	7.45^{b}	8.97^{ab}	9.77^{a}	8.28^{ab}	1.71*	7.20^{b}	10.27a	8.38^{ab}
NH3-N (mg/dl)	21.20	21.66	21.00	22.16	$0.89^{n.s}$	20.94	22.68	20.90

* G1= Control diet, G2= Control diet plus 34 mg Thiamin. G3= Control diet plus 34 mg Thiamin + 16g NaHCO3, G4 = Control diet plus 16g NaHCO3.
a,b,c,d means in the same row with different superscripts differed significantly at ($p<0.05$).
- Each value is amean of 12 samples

Table (3): Effect of diets supplemented with thiamin and/or sodium bicarbonate on nutrients digestibility and nutritive value on DM basis

Item	Experimental Groups*				SE
	G1	G2	G3	G4	
Digestibility%					
DM	80.71	79.25	80.78	81.42	0.92 n.s
OM	81.87	80.47	82.42	82.41	0.92 n.s
CF	70.95	70.04	70.41	70.19	0.4 n.s
CP	74.62[a]	72.27[ab]	67.53[b]	75.39[a]	3.54*
EE	50.76	45.31	48.76	49.85	2.38 n.s
NFE	88.69	87.52	90.38	89.53	1.22 n.s
Nutritive value%					
TDN	75.87	74.96	76.19	76.87	0.79 n.s
DCP	10.27[a]	9.94[ab]	9.29[b]	10.37[a]	0.49*

* G1= Control diet, G2= Control diet plus 34 mg Thiamin, G3= Control diet plus 34 mg Thiamin + 16g $NaHCO_3$, G4 = Control diet plus 16g $NaHCO_3$.

- a,b means in the same row with different superscripts differed significantly at ($p<0.05$).
- Each value is a mean of 12 samples

Table (4): Effect of diets supplemented with thiamin and/or sodium bicarbonate on some serum parameters of rams

Item	Experimental Groups*					Mean of sampling time		
	G1	G2	G3	G4	SE	0hr	4hr	SE
Total protein (g/dl)	6.19	6.33	6.76	5.34	0.84 n.s	6.53	5.78	1.1n.s
Albumin (g/dl)	4.19	3.96	4.06	3.63	0.34 n.s	4.14	3.78	0.5n.s
Globulin(g/dl)	2.00	2.36	2.70	1.70	0.61 n.s	2.39	2.00	0.55n.s
A/G ratio	2.32	2.55	1.85	2.59	0.48 n.s	2.48	2.18	0.43n.s
Urea(mg/dl)	34.30	35.84	35.74	33.80	1.45 n.s	36.38	33.46	4.14n.s
GOT (units/L)	47.66	59.26	59.98	48.45	9.46 ns	58.80	48.88	14.04n.s
GPT(units/L)	41.29	58.05	63.21	40.53	16.38 ns	56.87	44.63	17.27n.s
Glucose (mg/dl)	85.19	91.13	96.19	77.73	11.24 n.s	83.91	91.21	10.32n.s
Cholesterol (mg/dl)	363.30	363.88	320.25	366.25	31.33 n.s	355.09	351.75	4.73n.s

* G1= Control diet, G2= Control diet plus 34 mg Thiamin, G3= Control diet plus 34 mg Thiamin + 16g $NaHCO_3$, G4 = Control diet plus 16g $NaHCO_3$.

- Each value is amean of 12 samples.

supplemented with 34 mg thiamin (G2) or with thiamin plus $NaHCO_3$ (G3) resulted in significant ($p<0.05$) decrease of DCP comparing with the unsupplemented diet (G1) (Table 3) and the reasons are not clear.

Serum parameters

Concerning blood metabolites, data of Table (4) clearly showed that diets supplemented with 34 mg thiamin plus 16g $NaHCO_3$ (G3) resulted in increasing serum glucose ($P>0.05$) by 12.9% comparing with unsupplemented group (G1). Differences in serum glucose among G1, G2 and G4 were not significant ($P>0.05$). This result was in line with that obtained by Majee *et al.* (2003).

Moreover, no significant differences were detected among the four groups in the concentration of total cholesterol (Table 4).

With regard to nitrogen metabolites, it could be noticed that supplementation of thiamin or $NaHCO_3$ or both of them to diets of rams had no significant effect ($p>0.05$) on total protein, albumin, globulin, ratio of albumin and globulin and urea-N in the serum comparing with the control (Table 4). Result of urea reported here was in line with that of Grigat and Mathison (1982).

Obviously, data of Table (4) clearly showed that both GOT and GPT activity was increased insignificantly ($P>0.05$) by supplementing of thiamin alone (G2), or plus $NaHCO_3$ (G3) comparing with control diet (G1). no possible illustration is available. However, differences in GOT and GPT between (G4) (16g $NaHCO_3$ /h/d) and G1 was not significant ($p>0.05$).

CONCLUSION

From the previous results, it could be concluded that supplementation of thiamin as 34 mg /h/d with or without 16g $NaHCO_3$ /h/d significantly ($p<0.05$) increased the energy precursors (TVFA's) in the rumen.

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تأثير إضافة الثيامين مع / أو بدون بيكربونات الصوديوم على تخمرات الكرش و الهضم و وقياسات سيرم الدم فى الكباش

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إستخدم لهذه الدراسة (4)كباش، متوسط أوزانها (58)كجم، فى تجربة باستخدام المربع اللاتيني (4*4)، و كانت كل فترة 21يوما .وكانت الأربع معاملات هي التغذية على عليقة المقارنة)مجموعة (1، المجموعة (2)تغذى على عليقة كنترول مضاف إليها 34مللجرام ثيامين/رأس/يوم، المجموعة (3)تغذى على عليقة كنترول مضاف إليها 34 مللجرام ثيامين 16 +جرام بيكربونات الصوديوم/رأس/يوم، أما المجموعة الرابعة فتم تغذيتها على عليقة كنترول مضاف إليها 16جرام بيكربونات صوديوم/رأس/يوم .

أشارت النتائج إلى أنه لم تحدث أي معاملة من المعاملات السابقة أي تأثير معنوي على قيم الـ pHأو تركيزات الأمونيا بالكرش بينما أحدثت الإضافات في المجموعة الثانية و الثالثة زيادة معنوية)على مستوى معنوية (5%في تركيز الأحماض الدهنية الكلية الطيارة بالكرش مقارنة بمجموعة المقارنة .

أما نتائج معاملات الهضم فلم تختلف معنوياً بين المجاميع الأربعة فيما عدا معامل هضم البروتين حيث انخفضت معنوياً قيمة هذا المعامل فى المجموعة الثالثة)على مستوى معنوية .(5%

و قد أظهرت الإنزيمات الناقلة لمجمعة الأمين GOT)،(GPTفى سيرم الدم نشاطا مرتفعا بمعنوية)على مستوى (5%فى المجموعتين الثانية و الثالثة عنها فى المجموعة الأولى)مجموعة المقارنة (و المجموعة الرابعة .

ونستخلص من هذه الدراسة أن الثيامين بمفرده أو مع بيكربونات الصوديوم له تأثير على تخمرات الكربوهيدرات بالكرش)متمثلا فى زيادة كمية الأحماض الدهنية الطيارة الكلية فى سائل الكرش (بدون أى تأثيرات ضارة على صحة الحيوان.