

INFLUENCE OF SUPPLEMENTALSHEEP RATION WITH ZINC SOURCE (INORGANIC VS. ORGANIC) ON THEIR DIGESTIBILITY AND RUMINAL FERMENTATION.

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SUMMARY

This study was conducted to investigate the influence of zinc sulfate and zinc methionine supplementation on the nutrients digestibility, nutritive values of feeds and ruminal fermentation of sheep. Fifteen sheep averaged (52.2 ± 1.40 kg) were divided into five similar groups (three animals each), they were fed basal diet containing 33.34 mg Zn/kg dry matter (DM) with no supplemental Zn (control). Control group was consisted of concentrate feed mixture (CFM), corn silage and rice straw without zinc supplementation. The other four experimental groups supplemental with 30 or 60 mg of Zn/kg of DM from Zn sulfate (ZnS) or zinc methionine (ZnMet) to control diet. Results indicated that zinc addition either as zinc sulfate or zinc methionine increased ($P < 0.05$) the digestibility of all nutrients which were reflected on the nutritive values (as TDN and DCP) of diets. Addition of zinc sulfate or zinc methionine reduced ammonia-N and increased both TVFA's rumen volume, rumen digesta and microbial protein synthesis of sheep.

Key words: *Zinc sulfate, zinc methionine, digestibility, ruminal fermentation, sheep.*

INTRODUCTION

Zinc is an important trace element for animals, functioning largely or entirely in enzyme systems and being involved in protein synthesis, carbohydrate metabolism, and many other biochemical reactions (Rink and Kirchner, 2000). Zinc is associated with more than 300 enzymes either as component or as activator. Zinc is an essential trace mineral that serves some roles in an animal's body such as the controlling of growth by affecting on feed intake, secretion of mitogenic hormones, gene expression of proteins (Huerta *et al.*, 2002). The most widely used products for zinc supplementation are inorganic (zinc sulfate and zinc oxide), and recently, organically bound zinc supplements are being used in animal diets (Saeid *et al.*, 2010).

Use of organically complexed trace minerals can help prevent these losses, due to increased stability in the upper gastrointestinal tract of the animal. Indeed, a variety of trials have demonstrated greater bioavailability of organically complexed trace minerals, which in turn would allow for lower inclusion rates and reduced excretion (Bao and Choct, 2009). However, in recent years, there has been considerable interest in feeding ruminants amino-trace minerals to increase the bioavailability of the mineral above that of the soluble inorganic sources (Luo *et al.*, 1996). The metal complex or chelate is stable in the digestive tract and is thus protected from forming complexes with other dietary components which could inhibit the absorption (Cao *et al.*, 2000). However, Zn could be used to reduce ruminal degradability of feed protein and to promote greater quantities of ruminal escape protein (Britton and Klopfenstein, 1986).

The objective of this study was to compare the effect of Zinc sulfate and Zinc methionine supplementation of sheep on nutrient digestibilities, nutritive values and some ruminal fermentation.

MATERIALS AND METHODS

This study was conducted in El-Noubaria, Animal Production Research Station belonging to the Animal Production Research Institute, Agricultural Research Centre, Egypt. Fifteen sheep were divided into five similar groups (three animals each). Sheep Barki Rams were fed on one of the following ration: 1- Concentrate feed mixture (CFM), corn silage and rice straw without zinc supplementation (Control). 2- Same as one supplemented with 30mg zinc sulfate /kgDMI. 3- Same as one supplemented with 60mg zinc sulfate /kgDMI. 4-Same as one supplemented with 30mg zinc methionine /kgDMI. 5- Same as one supplemented with 60mg zinc methionine /kgDMI according to the feed allowances of NRC (1994). Feed additives (Zinc methionine with content of 80.5% methionine hydroxy analogue and 15.10% Zinc sulfate)and were mixed manually with some ground amounts of CFM(Multivita Company, 6th October city,Egypt).Ingredient and chemical composition of CFM, corn silage and rice straw are presented in Table (1).

Table (1): Proximate analysis of concentrate feed mixture (CFM, corn silage and rice straw fed to sheep on DM basis (%).

Item	CFM	Corn silage	Rice straw
DM	89.53	29.53	89.54
OM	93.83	93.84	90.63
CP	16.22	7.98	3.22
CF	8.46	23.27	31.45
EE	3.16	2.44	1.08
NFE	65.99	60.15	54.88
Ash	6.17	6.16	9.37
NDF	34.84	48.63	74.36
ADF	22.06	27.55	59.14
ADL	2.77	4.38	11.47
Non-fiber carbohydrate	39.61	34.79	11.97
Net energy of lactation (M cal/kg DM)	1.37	0.86	0.47
Calcium (g/kg of DM)	0.66	0.27	0.17
Phosphorus (g/kg of DM)	0.25	0.19	0.11
Zn (mg/kg)	41.7	21.2	16.7

*CFM contained Corn grain,(ground)40%, wheat bran 20.5 %, Soybean, meal 44%CP 16.5 %, Undecorticated Cottonseed meal5 %, Sunflower cake5.5 %, Sugar Beat pulp 6%, Molasses 3%, Limestone 2%, Sodium chloride 1.1% and mineral premix 0,4%

Digestibility and nitrogen balance trials:

Digestibility and nitrogen balance trials were carried out using fifteen Barki rams (52.2 ± 1.40 kg, in average) (three rams for each group). Each trial lasted for four weeks; the first three weeks were as a preliminary period, followed by one week for feces and urine collection. Sheep were fed twice daily at 8 am and 3 pm, water was offered freely. Each animal was offered the experimental rations according to NRC, (1994). Chemical composition of feeds, feces and urine were determined according to AOAC (2005) methods. Sub samples (20%) of feces and urine were taken once daily then stored at -18°C until analyses. Fecal samples were dried at 60°C for 72 hrs. Feed and fecal samples were ground through 1 mm screen on a Wiley mill grinder and a sample of 50 gm/ (ration/sheep) was taken for analysis. The samples of feed and

feces were analyzed for crude protein (CP), crude fiber (CF), ether extract (EE) and ash, while the urine samples were analyzed for nitrogen (N) content according to AOAC (2005). Cell wall constituents (NDF, ADF and ADL) were determined according to VanSoest (1991). Hemicellulose and cellulose were calculated by differences.

Rumen fermentation trials:

Samples of rumen liquor were taken at 0,1, 3 and 6 h post feeding from three ruminally-cannulated Barki ewes with approximately 42.5±0.5kg BW for each treatment, to be immediately analyzed for pH using Orion 680 digital pH meter. The rumen fluid samples were preserved for ammonia nitrogen (NH₃-N) determination according to Preston (1995). Concentration of total volatile fatty acid (VFA's) was estimated by using steam methods (Warner, 1964). Rumen volume was determined by colorimetric method of Cr-EDTA before, 3 and 6 hrs after feeding (El-Shazly et al. 1976). The microbial protein synthesized (gMP/day) in the rumen of sheep fed the experimental diets was calculated using the model equation by Borhami et al. (1992) as follow: g MP / day = mole VFA produced / day X 2 X 13.48 X 10.5 X 6.25 / 100.

where one mole VFA yield about 2 mole ATP (Walker, 1965), one mole ATP produce 13.48 YATP (g DM microbial cell); Borhami et al. (1979), N % of dry microbial cell = 10.5 (Hungate, 1965).

Statistical analysis:

Data were statistically analyzed as (2 x 2) factorial designs using two – way ANOVA design procedure of SAS (2006); the model describing each trait was assumed to be:

$$Y_i = \beta_0 + \beta_{1z_{1i}} + \beta_{2z_{2i}} + \beta_{3z_{1iz_{2i}}} + e_i$$

Where:

Y_i = outcome score for the i_{th} unit

β_0 = coefficient for the intercept

β_1 = mean difference on levels

β_2 = mean difference on zinc sources

β_3 = interaction of levels and zinc sources

z_{1i} = dummy variable for levels

z_{2i} = dummy variable for zinc sources

e_i = residual for the i_{th} unit

Separation among means was carried out by using Duncan's Multiple Range Test, (Duncan, 1955).

RESULTS AND DISCUSSION

Feed intake and Nutrient digestibilities:

The results presented in Table (2) showed that intake DM was insignificant ($P > 0.05$) between treatments. No significant differences of DM intake among treatments (Table 2). Miller *et al.* (1989) reported that supplementation of Zn (ZnSO₄) up to 2000 ppm of the diet did not influence DMI in dairy cows. These were also observed by Gaafar *et al.* (2011). However, daily Zn intake (33.34 up to 94.48) seems to be less than that was recommended by NRC (2001) (130 – 180 mg/day). In the same manner, Salama *et al.* (2003) noticed that supplementing dairy goats with 1 g/d ZnMet resulted in an increase of DM, CP and OM digestibility. They also reported that supplementing with 20 mg organic Zn improved NDF digestibility as a positive role in its effect in fiber digestion. In the meantime, Garg *et al.* (2008) found that supplementation with 20 mg organic zinc improved digestibility of both cellulose and ADF. However, the improvement in digestibility could be due to the direct role of ZnMet in stimulating anaerobic fermentation of OM which in turn could improve the efficiency of nutrients utilization. Data presented in Table (2)

indicated that supplemental 60 mg/kg DM zinc methionine significantly ($P<0.05$) increased the all nutrient digestibilities and cell wall constituents (NDF, ADF and ADL) compared to other groups. While supplemented of two different levels of zinc methionine significantly ($P<0.05$) increased the all nutrient digestibilities and cell wall constituents (NDF, ADF and ADL) compared to two different levels of zinc sulfate and control groups, Also, no significant differences were found between the two Zn sources of EE digestibility. The higher apparent digestibility coefficient with zinc supplemented rations may be due to the improvement of their digestibility and absorption. These results are in line with those obtained by Shakweer *et al.* (2005) who found that the apparent digestibility of DM, OM, CP and CF were significantly improved with added different levels of zinc methionine in the ration of Friesian dairy cows. Mandal *et al.* (2007) indicated that a diet containing about 32.5 mg Zn/kg DM was adequate to support normal growth and digestibility in bulls. Mousa and EL-Sheikh, (2004) found that the apparent digestibility of DM, OM, CP, CF; EE and NFE were slightly increased by different levels of zinc sulfate supplementation to the ration of lactating buffaloes. However, Dinn *et al.* (1998) reported that the apparent digestibility of CF was significantly increased with addition of protected amino acids than those of unprotected. Balabanova *et al.* (2011) indicated that the feeding organic forms of zinc a tendency to higher digestibility of crude protein, fat, crude fiber, nitrogen-free extracts and ash compared to inorganic forms of zinc. Also, the digestibility of the fiber was the most increased.

Table (2). Effect of different levels of zinc sulfate or zinc methionine supplementation on feed intake and digestibility coefficient in sheep.

Item	Control	Zinc supplement			
		Inorganic Zn		Organic Zn	
		Zinc Sulfate, 30mg/kg DM	Zinc Sulfate, 60mg/kg DM	Zinc Methionine, 30mg/kg DM	Zinc Methionine, 60mg/kg DM
Feed intake, (g/h/d)					
CFM	537.2	537.2	537.2	537.2	537.2
Corn silage	339.6	363.2	367.7	371.2	380.77
	±26.3	±20.6	±17.9	±28.5	±42.0
Rice straw	223.8	237.9	240	245.3	240.00
	±10.7	±19.5	±13.1	±9.4	±13.2
Total feed intake	1100.7	1137.7	1144.8	1153.7	1157.8
	±47.04	±34.0	±41.5	±27.2	±38.6
Zn conc.	33.34	64.06	94.20	64.37	94.48
Intake, mg/kg DM					
Digestibility (%)					
DM	56.44±1.07 ^d	59.72±0.83 ^c	60.33±0.66 ^c	61.65±0.19 ^b	62.38±0.28 ^a
OM	59.63±0.73 ^c	62.67±1.58 ^b	63.27±1.08 ^b	64.49±0.34 ^b	65.10±0.16 ^a
CP	63.24±0.66 ^c	65.68±0.47 ^b	65.88±0.72 ^b	67.14±0.26 ^a	67.69±0.43 ^a
CF	58.74±0.93 ^c	61.16±0.44 ^b	61.71±0.73 ^b	63.15±0.42 ^a	63.64±0.28 ^a
EE	69.86±0.53 ^b	72.41±0.74 ^a	72.52±0.92 ^a	72.39±0.68 ^a	72.08±0.55 ^a
NFE	58.83±1.07 ^c	62.19±0.47 ^b	62.93±0.89 ^b	64.10±0.10 ^a	64.79±0.68 ^a
Cell wall constituents (%)					
NDF	57.28±0.52 ^c	60.97±0.78 ^b	61.60±0.62 ^b	61.97±0.59 ^b	63.52±0.33 ^a
ADF	42.86±0.69 ^c	47.13±0.28 ^b	47.43±0.36 ^b	49.45±0.48 ^a	50.24±0.68 ^a
Lignin	31.88±0.19 ^c	36.06±0.55 ^b	37.11±0.83 ^b	38.83±0.44 ^b	39.95±0.27 ^a

^{a,b,c,d} means in the same row followed by different superscripts are significantly ($P<0.05$) different

Nutritive values:

Nutritive values expressed as TDN, DCP, DE and ME were ($P < 0.05$) increased by the supplementation of the two different levels of zinc methionine compared to the two levels of zinc sulfate and control groups (Table 3). The improved TDN and DCP might be due to the higher nutrients digestibility of zinc supplemented groups. These results are in accordance with Shakweer *et al.* (2005) and Mousa and EL-Sheikh (2004) who found that the TDN and DCP were significantly increased by added different levels of zinc methionine or zinc sulfate. However, the improvement in nutrients digestibility in relation to increase Zn supplementation level, was accompanied by the improvement in the nutritive value of the given diet expressed either as Kcal metabolizable energy (ME) or as digestible crude protein (DCP) (Roth *et al.*, 1992).

Table (3). Effect of different levels of zinc sulfate or zinc methionine supplementation on nutritive values in sheep.

Item	Control	Zinc supplement			
		Inorganic Zn		Organic Zn	
		Zinc Sulfate, 30mg/kg DM	Zinc Sulfate, 60mg/kg DM	Zinc Methionine, 30mg/kg DM	Zinc Methionine, 60mg/kg DM
TDN, %	57.76±0.21 ^c	60.65±0.44 ^b	61.17±0.61 ^b	62.32±0.22 ^a	62.90±0.74 ^a
TDNI, g	635.74±23.73 ^c	690.02±10.33 ^b	700.29±9.84 ^b	719.01±7.88 ^a	728.27±13.59 ^a
DCP, %	6.98±0.03 ^c	7.14±0.10 ^b	7.15±0.08 ^{ab}	7.25±0.11 ^a	7.32±0.09 ^a
DCPI, g	76.83±1.38 ^c	81.23±0.62 ^b	81.85±0.44 ^b	83.65±0.89 ^a	84.75±1.03 ^a
DE (M cal/kg)	2.55±0.05 ^c	2.67±0.02 ^b	2.70±0.05 ^{ab}	2.75±0.03 ^a	2.77±0.02 ^a
ME (Mcal kg ⁻¹)	2.09±0.04 ^b	2.20±0.03 ^a	2.21±0.06 ^a	2.26±0.04 ^a	2.28±0.06 ^a

^{a,b,c}: means in the same row followed by different superscripts are significantly ($P < 0.05$) different

Nitrogen utilization

The data of nitrogen intake in table (4) showed that no significant differences were found between the supplemental Zn and control groups of nitrogen intake. The presented results indicated that dietary the

Table (4). Effect of different levels of zinc sulfate or zinc methionine supplementation on nitrogen utilization in sheep.

Item	Control	Zinc supplement			
		Inorganic Zn		Organic Zn	
		Zinc Sulfate, 30mg/kg DM	Zinc Sulfate, 60mg/kg DM	Zinc Methionine, 30mg/kg DM	Zinc Methionine, 60mg/kg DM
Nitrogen intake (NI), g/d	19.43±0.74	19.80±0.48	19.87±0.52	19.94±0.57	20.04±0.68
Nitrogen absorbed (NA), g/d	12.29±0.33 ^b	13.01±0.49 ^a	13.09±0.13 ^a	13.39±0.11 ^a	13.56±0.17 ^a
Nitrogen retained (NR), g/d	4.29±0.22 ^c	4.96±0.15 ^b	5.16±0.10 ^b	5.13±0.17 ^b	5.57±0.21 ^a
N- retained e as % of N-intake	22.10±0.29 ^c	25.07±0.37 ^b	25.95±0.55 ^b	25.71±0.26 ^b	27.81±0.17 ^a
N- retained as % of N-absorbed	34.94±0.55 ^c	38.18±1.03 ^b	39.38±0.75 ^b	38.30±0.89 ^b	41.08±0.39 ^a
Zinc conc. In faces, mg/h/d	0.96	8.04	14.18	9.22	15.27

^{a,b,c}: means in the same row followed by different superscripts are significantly ($P < 0.05$) different

supplemental 60 mg/kg DM zinc methionine significantly ($P < 0.05$) increased the nitrogen retained (NR), N-retained as % of N-intake and as % of N-absorbed compared to other groups, while supplement of two different levels of zinc sulfate and zinc methionine significantly ($P < 0.05$) increased the nitrogen absorbed (NA) compared to control group. However, all rations were showed positive retained N. Differences in nitrogen retained values may be due to the differences in amino acids composition of protein sources and its

digestibility. The higher percentage of dietary nitrogen retained noticed with ration supplemental 60 mg/kg DM zinc methionine compared with the control, these may be due to the more digestible protein; it had more ($P<0.05$) nitrogen utilization, this finding was agreed with that of Kleinschmit *et al.*(2006). Dietary nitrogen utilization (% N retained of N-intake) was obviously higher ($P<0.05$) with ration supplemented with 60 mg/kg DM zinc methionine, than the all other rations.

Ruminal fermentation:

Ruminal pH and ruminal metabolites ($\text{NH}_3\text{-N}$ and TVFA: S) values were significantly affected by supplementation of zinc (Table 5). The pH value of rumen liquor were significantly ($P<0.05$) decreased by supplemental inorganic or organic zinc compared with control group. The pH values are within the normal range obtained by Van Soest (1982) who stated that the optimum pH value for growth of cellulytic microorganisms was 6.7 ± 0.5 pH degree. Supplemental with 60 mg/kg DM zinc methionine had significantly ($P<0.05$) decreased the $\text{NH}_3\text{-N}$ and rate of out flow compared to control group. However, supplementation with organic zinc had more proration than inorganic zinc in that concern. While, the concentrations of TVFA's, rumen volume, rumen digesta and microbial protein synthesis significantly increased ($P<0.05$) with 30 and 60 mg/kg DM zinc methionine compared 30 and 60 mg/kg zinc sulfate and control groups. This increase in TVFA's may be due to the increase of apparent digestibility of organic matter. These results suggested that the anaerobic fermentation of protected amino acids were more efficient and faster yielding more TVFA's than that in control and inorganic supplementation. The decrease of ruminal $\text{NH}_3\text{-N}$ concentration with zinc methionine supplementation may be due to improve of the rumen microbe's activity utilizing $\text{NH}_3\text{-N}$ to produce microbial protein, in the meantime, improve degradation of feed utilization (Froetschel *et al.*, 1990). Hideaki *et al.* (2005) found that feeding zinc methionine to Japanese black steers was more effective in ruminal fermentation than inorganic zinc. Spears *et al.* (2004) showed that total VFA concentrations were higher ($P<0.05$) in steers receiving ZnGly or ZnMet than in those fed the control and ZnSO_4 treatments. Increased acetate and decrease butyrate with ZnMet is consistent with improved microbial efficiency of energy utilization (France and Siddons, 1993). Arelovich *et al.*(1998) reported that concentration of $\text{NH}_3\text{-N}$ was decreased by added Zn. This might be due to that zinc sulfate depress urease activity directly or it might inhibit growth and reduce the population of ureolytic bacteria (Arelovich *et al.*, 2000). Shakweer *et al.* (2006) reported that the increase proportion of propionate in ruminal VFA's leads to an increase in energetic efficiency of ruminal fermentation which may explain the consistent benefits obtained from addition of chelated zinc.

Table (5). Effect of different levels of zinc sulfate or zinc methionine supplementation on someruminal fermentation of sheep.

Item	Control	Zinc supplement			
		Inorganic Zn		Organic Zn	
		Zinc Sulfate, 30mg/kg DM	Zinc Sulfate, 60mg/kg DM	Zinc Methionine, 30mg/kg DM	Zinc Methionine, 60mg/kg DM
pH	6.69±0.02 ^a	6.55±0.01 ^b	6.47±0.01 ^c	6.48±0.01 ^c	6.45±0.01 ^d
Ammonia nitrogen concentration (mg/100 ml)	15.05±0.13 ^a	12.70±0.17 ^b	12.55±0.15 ^b	12.29±0.08 ^c	12.16±0.13 ^c
Total Volatile fatty acids concentration(TVFA) (mmol/100 ml)	10.46±0.13 ^c	11.46±0.05 ^b	11.55±0.12 ^b	11.83±0.09 ^a	12.05±0.11 ^a
Rumen volume (L)	3.08±0.15 ^c	3.65±0.12 ^b	3.72±0.06 ^b	3.94±0.07 ^a	4.05±0.11 ^a
Acetate	54.64±0.36 ^b	57.59±0.57 ^a	58.13±0.38 ^a	58.16±0.32 ^a	58.44±0.29 ^a
Propionate	25.77±0.36 ^a	24.37±0.26 ^b	24.25±0.23 ^b	23.86±0.07 ^c	23.10±0.43 ^c
Butyrate	10.96±0.45 ^a	6.41±0.19 ^b	5.24±0.06 ^c	5.10±0.10 ^c	4.30±0.22 ^d
Rate of out flow (% hr)	7.29±0.06 ^a	5.65±0.04 ^b	5.60±0.07 ^b	5.22±0.05 ^c	5.10±0.14 ^c
Rumen digesta (kg)	3.48±0.25 ^c	4.13±0.13 ^b	4.25±0.07 ^{ab}	4.32±0.02 ^a	4.43±0.11 ^a
Microbial protein synthesis (MP, g/d)	40.87±1.08 ^d	62.57±1.04 ^c	70.76±2.61 ^b	74.05±3.62 ^b	79.20±0.53 ^a

^{a,b,c,d:} means in the same row followed by different superscripts are significantly ($P<0.05$) different

CONCLUSION

From the present study it can be concluded that dietary supplement either 30 or 60 mg Zn to the diet, showed beneficial supplement effects to improve nutritive value and ruminal activity of ram barki.

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تأثير مصدر الزنك (الغير عضوى مقابل العضوى) والمضاف للعلائق على الهضم و تخمرات الكرش للاغنام

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نفذت هذه الدراسة بهدف التحقق من تأثير اضافة كبريتات الزنك و الزنك مثنونين على هضم المكونات الغذائية و القيمة الغذائية للاعلاف و تخمرات الكرش فى الاغنام. تم تقسيم خمسة عشر من الاغنام الى خمسة مجاميع متساوية (ثلاثة فى كل مجموعة) و تم تغذيتهم بالعليقة الكنترول 33.34 بدون اى اضافة للزنك. العليقة الكنترول كانت تتغذى على مخلوط العلف المركز وسيلاج الذرة و قش الارز بدون اضافة للزنك. الاربعة علائق التجريبية الاخرى تم امدادهم ب30 او 60 ملجم / كجم مادة جافة من كبريتات الزنك او الزنك مثنونين. النتائج اشارت الى ان اضافة كبريتات الزنك او الزنك مثنونين زاد من معاملات هضم المكونات الغذائية بصورة معنوية و الذى انعكس على القيمة الغذائية للاعلاف بينما اضافة كبريتات الزنك او الزنك مثنونين قلل من تركيز الامونيا وزياده تركيز كلا من الاحماض الدهنية الطيارة وكميه البروتين الميكروبي المخلق بالكرش فى الاغنام.