INFLUENCE OF DIETARY SUPPLEMENTATION OF VARIOUS SELENIUM SOURCES ON NUTRIENT DIGESTIBILITY, GROWTH PERFORMANCE AND BLOOD METABOLITES IN MALE BUFFALO CALVES

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(Received 17/5/2017, accepted 1/7/2017)

SUMMARY

An experiment was conducted to compare the effect of organic (selenized yeast) and inorganic (Na-selenite) selenium on nutrient digestibility, growth performance and blood parameters of growing buffalo claves. Fifteen Egyptian healthy male buffalo calves were divided randomly into three groups (5 animals each). The treatment groups were as follows: control group (G1) fed basal diet without selenium supplement and treated groups fed 0.22 mg Se/kg DM as either Na –Selenite (G2) or selenized yeast (G3) to the concentrate mixture. All animals were fed 70% of their requirements as concentrate mixture, while wheat straw given as roughages *ad libitum* The results indicated that there were significant ($P<0.05$) improvements in the digestibility coefficient of organic matter, crude protein, either extract and crude fibre due to dietary supplementation of both Se- yeast and Na-Selenite. No significant differences were observed in growth rate and total dry matter intake of calves between treatments groups. Dietary Se-yeast and Na-selenite supplementation led to significantly ($P<0.05$) improved fed conversion ratio for calves as compared with control group (10.86 and 11.09 vs. 11.99). Calves fed Se-yeast or Na-Selenite supplement had increased ($P < 0.05$) concentrations of selenium in whole blood, greater activity ($P < 0.05$) of blood glutathione peroxidase (GSH-Px) with lower ($P < 0.05$) cholesterol and urea concentration than those fed the control diet. In conclusion, dietary supplementation with Se-yeast was more effective than sodium selenite in improving nutrient digestibility, feed conversion efficiency and increasing both Se concentration and GSH-Px activity in the blood of buffalo calves.

Key words: *Selenium sources; growth performance; nutrient digestibility; buffalo calves*

INTRODUCTION

Selenium (Se) plays important roles in several metabolic process including antioxidant defense systems, thyroid hormone metabolism and immune function (Brown and Arthur 2001). The low selenium level or low bioavailability in soils is reflected in naturally low selenium content of feedstuffs results in animals being deficient in this element (Johnson et al. 2010). The absorption of selenium in ruminants was limited because its mineral compounds are strongly reduced to unavailable forms by rumen bacteria (Pavlata et al., 2002). The factor that reduces the absorption is low rumen pH, especially the high-yielding cows that receive high doses of energy feed. In addition, some elements extremely reduce selenium absorption (*e.g.* magnesium); however, vitamins C and E facilitate its absorption (Kondracki and Bednarek 1996).

The Federal Drug Administration (FDA) in 1987 was amended the dose of Se supplement to ruminant diets from 0.1 ppm at that time to 0.3 ppm at time. In September 2003 the FDA, was modified again to permit the use of selenium yeast (Se-yeast) in diets for dairy and beef animals instead of sodium selenite and selenate.

Selenium supply to ruminant diets in the inorganic or organic form. Sodium selenite is most frequently applied in the feed industry, which are commonly offered in mineral premixes or injected. Selenized yeast (Se-Y) is the traditional form of supplying organic selenium, this product grown in high

selenium medium. Yeast contains selenium mostly as selenomethionine and also has a major amount of selenium in other forms. The dietary inclusion of selenomethionine lead to more retention of Se in ruminants and much longer than Se from inorganic sources. However, the use of Se-yeast in animal feeds is less favorable, because it is relatively expensive.

Numbers of studies on calves were reported the immune mechanism was improved through proper nutrition. Since selenium plays a significant role in immune mechanism and growth. In addition, organic selenium supplementation could be beneficial to protect the pre ruminant calves against infection and to alleviate the weaning stress, thereby enhancing the production performance. Inclusion of 0.3 ppm Se in the diet of buffalo calves significantly enhanced their immune status (Shinde et al., 2009)

The current study was carried out to compare the effect of organic (Se-yeast) and inorganic (Na-Selenite) selenium on nutrient digestibility, growth performance and blood parameters of growing buffalo claves.

MATERIALS AND METHODS

Animals, rations and management

The experiment was conducted at the research farm of Faculty of Agriculture, Al-Azhar University, Assiut branch, Egypt. Fifteen healthy male buffalo calves of 13-14 months of age weighing 152.33 ± 20.61 kg were divided randomly into three groups of (5 animals each). Group 1 was considered as a control and fed a basal diet consisting of roughage and concentrate mixture without any selenium supplementation. Group 2 fed the basal diet supplemented with 0. 22 mg Se/kg DM as sodium selenite (Na -Selenite), while Group 3 received the same basal diet that supplemented with 0.22 mg Se/kg DM as selenized yeast. The basal diet contains 0.05 mg/kg DM Se, and the doses of Se were dietary supplemented to the ration. The source of selenized yeast is (Shandong Long live Bio-technology Co., Ltd. China), while the source of sodium selenite is from (Loba Chemie Pvt.Ltd.107, Wode house Roadm, Mumbai 400005, India). The ingredients of concentrate mixture were 10 % corticated cotton seed meal, 10% soybean meal, 20% wheat bran, 56% yellow corn, 1% vitamin, 2% limestone, 1% sodium chloride. The chemical composition of concentrate mixture is clear in table (1).

The animal's requirements for CP and TDN were calculated according to NRC (2001). All calves of three groups were fed 70% of their requirements as concentrate mixture while wheat straw was given *ad libitum*. The quantity of concentrate mixture was adjusted every month according to change in body weight. The animals were randomly allotted to experimental diets**.**

Rations were offered twice a day and the feed orts were weighed daily through the experimental period and actual feed intake was calculated. Feed conversion ratio was calculated and expressed in terms of kg dry matter (DM) per one-kg body weight gain. Diets were mixed daily and fed twice a day. Animals were housed in a well-ventilated animal shed with cemented floor and provided with feed and water individually. Before morning feeding the calves were weighed at the start of experiment and then every month to determine the changes in the body weight (BW). Clean drinking water with no detectable amounts of Se was provided *ad libitum* twice (at 10 and 15 h). Calves were fed their respective treatment diets for a period of 166 days.

Table (1): Chemical composition of concentrate mixture.

Chemical analysis	%
DM	91.42
OM	92.53
CP	15.47
TDN	63
CF	15.56
EE	2.94
NFE	58.56
Ash	9.96
Se /mg/kg DM	0.05

DM, dry matter; OM, organic matter; CP, crude protein; CF, Crude fiber; EE, ether extract; NFE, Nitrogen free extract.

Digestibility trials

Nutrients digestibilities were carried out using chromic oxide as indicator. Each trial lasted 5-days as preliminary period followed by a 7-day as collection period. The total rations contained approximately 0.5% Cr2O3 which has been mixed with the concentrate portion. Twice fecal grab were directly taken from the rectum approximately 200 g of fresh feces during each 24-hr period and composited by animal at the end of the 7-day collection period. Feces were mixed thoroughly and 20% of it was sampled and dried at 55°C in forced air oven. The dried fecal samples from each animal were grounded through 1mm mill screen openings and were saved for chemical analysis.

Chemical analysis and digestion coefficients measurements

The chemical analysis of feeds and faeces were accomplished according to the procedures of Association of Official Analytical Chemists AOAC, (1999) by using duplicate samples. Chromic oxide was analyzed by atomic absorption spectrophotometer by the methods described by Williams et al (1962). The apparent digestion coefficients of nutrients were calculated by expressing the difference between the content of nutrient in both consumed feed and faeces as a percentage of its intake, according to Van Keulen and Young (1977)

Blood sampling

Blood samples were collected from the jugular vein monthly after the morning feeding. Blood samples were divided in two parts; the first part was taken to measure Se concentration and GSH-Px activity. While, the second part was immediately centrifuged at 3000 rpm for 20 min. and serum was stored at -20 ºC until analysis. Concentration of serum total protein (TP), albumin (AL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol were determined by spectrophotometer (Unico, USA) using commercial test kits. Serum globulin (GL g/dl) was obtained as the difference between the total protein and albumin concentration. The concentration of Se in whole blood and diets was determined by atomic absorption spectrometry (Norheim and Haugen, 1986). Samples were prepared by oxidative digestion in a mixed solution with concentrated nitric and perchloric acids, using an automated system. The activity of GSH-Px was determined in whole blood of calves with commercial test kits. Method for determination of GSH-Px is based on catalytic oxidation of glutathione by hydroxide peroxide, and spectrophotometer (UV/VIS JENWAY 6305) used for reading as described by Sankari (1985).

Statistical analysis

Statistical analysis was done according to general linear model (G.L.M) of S.A.S program (2001), version 8.2. Differences between groups in nutrient digestibility coefficient, blood metabolites and growth performance data were evaluated by one-way ANOVA. Duncan Multiple Range Test (Steel and Torrie, 1980) was used to test the effect of treatments. The data were presented as mean ± S.E.M. Level of significance was set at $P<0.05$. Statistical model as follow:

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

Where: Y_{ij} = The observation ij, μ =The overall mean, T_i = The effect due to treatment i., E_{ij} = The experimental error.

RESULTS AND DISCUSSION

Nutrients digestibility coefficient

The average values of nutrients digestibility are presented in table (2). The organic matter (OM), crude protein (CP) and either extract (EE) digestibility coefficient were significantly ($P<0.05$) improved for calves fed supplemented Se-yeast (G2) and Na-Selenite (G3) than control group (G1). Also, crude fibre (CF) digestibility coefficient was improved ($P<0.05$) in Se-yeast group more than Na-Selenite and control groups. The increased nutrient digestibility in Se-yeast treatments suggests positive impacts of Se-yeast on rumen microorganisms rather than the host as previously illustrated by Alimohamady *et al*. (2013). In addition, this finding was in agreement with those found by Saleem (2016) he revealed that digestibility of DM, OM, CP, neutral detergent fiber (NDF) and acid detergent fiber (ADF) improved for lambs fed on Se-yeast supplementation as compared with control lambs. Also, Shi et al (2011) stated an

improvement in total digestibility of dry matter, crude protein, ether extract and NDF due to increase in Se level from 0.15 to 0.45 mg Se/kg DM in male goats.

Table (2): **Effects of dietary Se source on nutrient apparent digestibility coefficient and feeding value of rations.**

Item	Treatment			P- value
	Control	Se-yeast	Na-Selenite	
Apparent digestibility coefficient				
DM	$60.50^{b} \pm 0.79$	$67.90^{a} \pm 1.16$	$65.26^{a} \pm 0.33$	0.001
OM	$62.35^{b} \pm 0.85$	$65.86^{a} \pm 0.54$	$65.53^{a} \pm 0.87$	0.033
CP	$70.26^{b} \pm 1.06$	$77.14^{a} \pm 0.30$	$75.30^{a} \pm 0.17$	0.001
CF	$51.87^{b} \pm 1.02$	$57.72^{a} \pm 1.65$	$52.52^{b} \pm 1.29$	0.041
EE	$64.96^{b} \pm 0.24$	$74.06^{a} \pm 1.29$	$71.97^{a} \pm 1.28$	0.002
NFE	65.34 ± 0.57	64.13 ± 2.12	66.79 ± 0.75	0.426
Feeding Value				
TDN	61.50 ± 1.56	63.37 ± 1.56	63.70 ± 0.62	0.307
SE	51.58 ± 0.33	53.35 ± 1.55	53.70 ± 0.62	0.331
DCP	7.05 ± 0.07	7.18 ± 0.21	7.28 ± 0.09	0.528

$^{a, b}$: Means of the same row in each item with different superscripts are significantly different (P<0.05) for treatment effect.

DM, Dry matter; OM, Organic matter; CP, Crude protein; CF, Crude fiber; EE, Ether extract; NFE, Nitrogen free extract; TDN, Total digestible nutrients; SE, Starch equivalent; DCP, Digestible crude protein.

The feeding value in terms of total digestible nutrients (TDN), starch equivalent (SE) and digestible crude protein (DCP) was not affected with supplemented Se-yeast and Na-Selenite to claves diets. Nicholson *et al*. (1991) found no effect with Se supplementation to all ration of lambs and buffalo calves on nutritive value of ration (TDN and DCP). However, Taheri *et al*. (2016) reported that organic selenium seems to be a better choice, considering the nitrogen and energy available for metabolism and production in native goats.

Growth performance

Results presented in table (3) showed that the differences among all groups in body weight gain and daily weight gain were not significant .These findings are in line with those obtained by Mateo *et al*. (2007). Vinu *et al* (2012) found that supplemented Se-yeast at rate 0.3 ppm to calves diets did not reveal any significant difference on daily body weight gain as compared with control. Similarly, Lawler *et al.* (2004) showed that the growth performances of bulls in growing and finishing cattle were not affected by supplement different sources of Se. Also, the lack effect of Se on growth rate of finishing lambs fed 0.3 mg Se-yeast/kg DM was conducted by Dominguez-Vara *et al.* (2009).

Table (3): Growth Performance of buffalo calves fed different sources of selenium.

*Item	Treatment			P- Value
	Control	Se-yeast	Na-Selenite	
Initial weight (kg)	152.50± 24.48	152.00 ± 15.21	152.50 ± 22.13	0.999
Final weight (kg)	265.75 ± 26.68	275.00 ± 25.42	273.75 ± 25.89	0.963
BW gain (kg)	113.25 ± 3.01	123.00 ± 12.73	121.25± 5.01	0.695
Daily gain (kg)	0.682 ± 0.031	0.741 ± 0.076	0.730 ± 0.03	0.695
Feed Intake (FI, kg/day)				
DMI of concentrate	5.22 ± 0.086	5.14 ± 0.079	5.18± 0.083	0.808
DMI of wheat straw	2.96^{a} ±0.016	$2.91^{b} \pm 0.010$	2.91^{b}± 0.010	0.003
Total DM intake	8.18 ± 0.089	8.05 ± 0.082	8.09± 0.083	0.541
Feed conversion ratio kg DM intake/kg gain)	$11.99^{a} \pm 0.13$	$10.86^{b} \pm 0.11$	$11.09^{b} \pm$ 0.11	0.001

**BW, Body weight; DMI, Dry matter intake*

$^{a, b}$:Means with different superscripts within a row are significantly different at (P < 0.05) for treatment effect.

Also, the results indicated that there were no significant differences in total dry matter intakes (DM) among all treatments groups (Table3). On the other hand, buffalos fed rations supplement with Se-yeast and Na-Selenite were significantly (P<0.05) higher for dray matter intake of wheat straw than fed control ration. The disappearance of the effect ether organic or nonorganic Se on feed intake and body weight gain of calves buffalos may be due to that Se requirements of the animals involved in our trial were so far covered by Se amount as analyzed in the basal diet (Alimohamady *et al*., 2013). However, these results agreement with findings of Vinu *et al*. (2012) reported that the total DM intake of calves not significant affected with supplemented 0.3 ppm organic selenium as compared with control one. Similarly, Juniper *et al.* (2008) observed no effect of different dietary levels of selenium (0.2 to 6.74 ppm) as selenium yeast on the dry matter intake on ruminant.

Data in Table (3) illustrated that supplement Se-yeast and Na-selenite to rations was improved significantly (P <0.05) feed conversion ratio of growing buffalos. This may be due to positive effect of selenium source on nutrient digestibility or due to the tendency increase on daily body weight gain of supplements groups as it is clear in (Table3). The improvement of feed conversion ratio was previously observed with Se sources supplemented to lambs by Saleem (2016) and Grace and Knowles (2002). In contrast, Juniper *et al.* (2008) observed no treatment effect on the feed efficiency in calves and lambs when supplemented with 5.86 and 6.63 ppm of selenium as selenium yeast .Also, Richards et al (2011) reported no significant effect in the feed gain ratio on dietary supplementation of 0.34 ppm of selenium yeast in beef cattle.

Blood metabolites

The data of serum parameters are summarized in Table (4). Supplemented Se-yeast and Na-Selenite to claves rations not effected on total protein, but decreased significantly (P <0.05) albumin and albumin \ globulin ratio as compared with control group. However, the value of globulin was significantly (P <0.05) higher in supplement groups than control one. The low values of plasma albumin in supplement groups lead to increase the level of plasma globulin in these groups as compared with control group. The higher value of globulin found with supplement Se groups may be due to the effect of Se supplementation, which increase significantly (p<0.05) total serum globulins, specificity γ -globulins as comparing with control (Hamam and abou zeina, 2007). Similarly, Shinde et al. (2009) found that supplementation of selenium and Vitamin E in the diet of buffalo calves had no effect on serum total protein.

Table (4): Effects of dietary Se source on some serum and whole blood metabolites

Item	Treatment			P- Value
	Control	Se yeast	Na-selenite	
Total protein (g/dl)	6.73 ± 0.08	7.03 ± 0.11	6.84 ± 0.11	0.126
Albumin (g/dl)	4.03^{a} ±0.17	3.57^{b} ± 0.04	3.36^{b} ± 0.11	0.003
Globulin (g/ dl)	2.71^{b}± 0.24	3.46^{a} ± 0.11	3.48^{a} ± 0.15	0.007
al/glo ratio	1.64^{a} ± 0.22	1.04^{b} ± 0.04	0.99^{b} ± 0.07	0.004
ALT (U/l)	25.33^{a}± 2.41	16.77^{b} ± 1.06	20.89^{ab} ± 1.41	0.007
AST (U/l)	20.33 ± 1.91	23.22 ± 1.96	23.89 ± 1.60	0.359
Cholesterol mg/dl	204.21^{a} ± 7.38	174.01^{b} ± 2.32	182.86^{b} ± 4.43	0.001
Urea (mg/ dl)	37.19^{a} ± 2.89	23.23^{b} ± 0.75	28.78^{b} ± 1.46	0.001
Se (µg/ l)	76.04^{c} ± 1.98	138.31^{a} ± 2.94	120.18^{b} ± 2.60	0.001
GSH-Px (U/l)	336.14^{c} ± 4.75	606.16^{a} ± 6.60	482.98^{b} ± 8.28	0.001

$^{a, b,c}$:Means of the same row in each item with different superscripts are significantly different (P<0.05) for treatment effect.

The value of ALT in Table 4 was significantly (P < 0.05) decreased for claves group fed supplement to organic Se as compared with control group. However, the values of AST were not apparently affected by Se supplementation. Alimohamady et al. (2013) found that selenium source had no significant effects on serum alkaline phosphatase (ALP), creatine phosphokinase (CPK), and aspartate aminotransferase (AST) activity. Also, Pond et al. (1995) noticed that dietary supplementation with either inorganic or organic Se to the basal diet of goats did not make any differences in serum AST and ALT.

The total cholesterol and blood urea concentration were significantly (P < 0.05) decreased with supplement Se-yeast and Na-selenite as compared with control. This results agreement with those reported previously by Slavik et al. (2008)

The results indicate that the supplement Se either inorganic or organic source were significantly ($P < 0.05$) increase the value of Se in whole- blood as compared with control group (Table 4) . Also, the calves received Se-yeast was significantly ($P < 0.05$) higher of Se concentration on whole-blood than that received Na-selenite. The increase in the mean blood Se concentration of calves received Se-yeast agrees with found by Qin *et al.* (2007) who found that organically bound Se can readily improve an animal's Se status and is superior to inorganic Se. Saleem (2016) indicated that lambs received Se in inorganic and organic source was significantly higher in blood Se concentrations than control groups. Weiss (2003) found that the concentration of Se in blood was increased at a rate 18% in cows received Se yeast than those received the same amount of sodium selenite. On other side, Esterhuyse (2012) stated that inorganic Se can contribute in improving Se status of animals.

Also, the results showed that supplement Se-yeast and Na-Selenite to buffaloes calves ration significantly ($P < 0.05$) increased whole blood GSH-Px as compared with control group (Table 4). The highest values of GSH-Px concentrations were recorded for Se-yeast group, followed by Na-selenite group, while the lowest value was found in the control group. Our results prove the positive correlation between blood Se concentration and blood GSH-Px activity. Correlation between Se concentration and GSH-Px activity was observed also by other studies Pavlata et al. (2001) in cattle, Pavlata et al. (2011) in goats, Rock et al. (2001) in lambs, Panev et al. (2013) in sheep. Similarly, Faixova et al (2007) found that lambs fed additional Se-enriched yeast had high level of Se in plasma, bigger activity of blood glutathione peroxidase ($P < 0.001$) and lower serum activity of creatine kinase.

CONCLUSION

From the present study, it can be concluded that selenium supplementation in form of organic and inorganic source improved nutrients digestibility coefficients and fed conversion ratio of buffalo calves, but Se-yeast was more effective than sodium. Both Se sources increased Se concentration and GSH-Px activity in the blood of buffalo calves.

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تاثير اضافه مصادر مختلفة من السلينوم على هضم المكونات الغذائيه ، اداء النمو وخصائص الدم فى ذكور عجول الجاموس

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اجريت هذة التجربه لمقارنه تاثير كل من السلينوم العضوى (سيلينات الخميرة) غير العضوى (سيلينات الصوديوم) على هضم المكونات الغذائيه ، اداء النمو ، خصائص الدم لعجول الجاموس الناميه. خمسه عشر من ذكور الجاموس الناميه تم تقسيمها بصورة عشوائيه الى ثلاث مجاميع (خمسه حيوانات بكل مجموعه). المجموعه الاولى تعتبر مجموعه المقارنه وهى التى تم تغذيتها على العليقه الاساسيه. المجموعه الثانيه تم تغذيتها على العليقة الاساسيه مضافا اليها 0.22 مجم سليلنيوم / كجم مادة جافه كسيلينات الصوديوم بينما المجموعه الثالثه تم تغذيتها على العليقة الاساسيه مضاف اليها 0.22 مجم سليلنيوم / كجم مادة جافه كسيلينات الخميرة. جميع الحيوانات تم تغطيه 70% من احتياجتها من المخلوط المركز بينما غذيت على تبن القمح كمادة خشنه حتى الشبع. اشارات الدراسه الى انه يوجد تحسين معنوى فى هضم كل من المادة العضويه ، والبروتين الخام ، مستخلص الاثير و الالياف الخام نتيجه اضافة كل من سيلينات الخميرة وسيلينات الصوديوم الى العليقة. لم تلاحظ اى اختلافات معنويه لكل من معدل النمو والغذاء الكلى الماكول بين المجموعات المختلفة. اضافة السلينوم فى صورته العضويه (سيلينات الخميرة) او الغير عضويه (سيلينات الصوديوم) حسن من الكفاءة الغذائيه للعجول بالمقارنه بمجموعه المقارنه. العجول المغذاة على سيلينات الخميرة وسيلينات الصوديوم زاد بها معنويا كل من تركيز السيلينوم فى سيرم الدم والجلوثين بيروكسيديز مع انخفاض معنوى لكل من تركيز االكوليسترول واليوريا فى سيرم الدم مقارنه بمجموعه المقارنه. نستنج من هذة الدراسه ان اضافه السلينوم فى صورته العضويه (سيلينات الخميرة) كان اكثر تاثيرا من سيلينات الصوديوم مما ادى الى تحسين هضم المكونات الغذائيه والكفاءة الغذائيه وزيادة تركيز السلينوم فى سيرم الدم ونشاط GSH-Px فى دم عجول الجاموس.