The effects of organic selenium (Sel-Plex) on the viability of pneumonic Holstein suckling calves

Ebrahimi, M.*; Towhidi, A.; Ganjkhanlou, M. and Amini, M.

Department of Animal Sciences, Faculty of Agriculture Science and Engineering, University of Tehran, Karaj, Iran.

Key Words:
Calf; glutathione peroxidase activity; IgG; selenium; weight gain.

Correspondence
Ebrahimi, M.
Department of Animal Sciences, Faculty of Agriculture Science and Engineering, University of Tehran, Karaj, Iran
Tel: +98(261)2248082
Fax: +98(261)2246752
Email: mrzh_ebrah@yahoo.com

Received: 06 February 2011,
Accepted: 21 June 2011

Abstract

The objective of this study was to determine the effects of supplemented selenium (Se) on plasma cortisol, red blood cell glutathione peroxidase activity (RBC GSH-PX-1), concentration of immunoglobulin G (IgG) and body weight in pneumonic suckling calves. Ten Holstein suckling male calves were randomly allocated to two groups and fed either unsupplemented milk (control) or with milk supplemented with 0.3 mg/kg DM Se (Sel-Plex) for two months. Sel-Plex had no significant effect on cortisol plasma concentration. A significant increase in red blood cell GSH-PX-1, body weight and serum concentration of IgG was observed in the treated group, along with a non-significant increase in body weight. The results indicate that Sel-Plex supplementation of suckling calves with a marginal selenium status increases GSH-PX-1, serum concentration of IgG and body weight of calves.

Introduction

Nutritional degenerative myopathy (white muscle disease) and poor growth rate in calves are common manifestations of selenium (Se) deficiency (Underwood, 1977). As an integral part of the enzyme glutathione peroxidase (GSH-Px), Se functions to prevent oxidative damage to body tissues (Hoekstra, 1974). Selenium deficiency results in reduction of neutrophil function, antibody production, proliferation of T and B cells and cytodestruction (Kiremidjian and Stotzky, 1987).

It has also been observed that plasma concentrations of immunoglobulin G (IgG) were higher in pregnant cows and their calves when the cows were supplemented with selenium which shows improvement in humoral immunity (Awadeh *et al*., 1998).

Since cortisol is an index of stress, plasma cortisol was evaluated to examine the effect of Sel-Plex on stress (Davis and Drackley, 1998). Whole blood Se concentration was found to be higher in calves fed organic selenium instead of selenite (Weiss, 2003), so we therefore used Sel-Plex as a source of organic selenium.

In most studies (Swecker *et al*., 1995; Awadeh *et al*., 1998; Gunter *et al*., 2003) prenatal supplementation of Se has been used to protect calves from Se deficiency, but in this study we directly supplemented calves with Sel-Plex in order to see its direct effects on calves which was born with marginal selenium status. Accordingly, the objectives of the current study were to determine the effect of milk supplemented with Sel-Plex on plasma cortisol (as stress status), red blood cell glutathione peroxidase activity (RBC GSH-PX-1), (as antioxidant status), concentration of immunoglobulin G (IgG), (as humoral immunity index) and body weight (BW); therefore, viability of Holstein suckling calves.

Materials and Methods

Animals and Location

In February 2006, ten Iranian Holstein male calves, approximately one month old, were selected from the dairy herd belonging to the department of Animal Science, University of Tehran, Karaj (35º48'N, 51º2'E). The calves (average BW (±Standard error) 47.16 ± 2.35 kg) were randomly allocated to one of two groups (n = 5) and kept in individual calf pens inside a building. During the experiment, all calves were suffering from pneumonia (*Pasteurella multocida*) that was prevalent at the time, but were under clinical examination by a veterinarian.

Experimental design

Calves had received ad libitum starter with either unsupplemented milk or milk supplemented with 0.3 mg/kg Se as Sel-Plex (Alltech Inc., Nicholasville, KY, USA) for two months. Milk intakes were set at 10% of BW and were adjusted weekly based on BW. Dry matter intake was calculated and 0.3 mg Sel-Plex per kg dry matter was added to the milk of suckling calves, according to the manufacturer's instructions. All calves had ten days' adaptation before the experiment began. The calves had ad libitum access to water. The starters (which were consumed by two groups) were formulated

to meet the calves' need for energy, protein, vitamins, and all minerals except Se (NRC 2001), Table 1. Dry matter intake of starter and milk was recorded daily for each calf during the experiment. Body weight was registered weekly at 9:00 am, after a 12h feed and water restriction. Ambient temperature and relative humidity were recorded at the time of blood sampling and every four hours during the experiment. Lower critical temperatures were also calculated by using the age of calves on the days of blood sampling (Gonzalez-Jimenez and Blaxter, 1962), (Table 2).

Blood collection

Blood samples were collected weekly from all calves, after feeding at 9:00am, through the jugular vein using heparinized tubes, and then kept at 4˚C. Samples were centrifuged (1,000 g for 20 min at 4ºC), and the separated plasma was stored at -20ºC until hormone analysis. On day 60 of the experiment, whole blood samples were obtained and heparinized once again. For analysis of red blood cell glutathione peroxidase activity (RBC GSH-PX-1), whole blood samples were stored at -80ºC and red blood cells were separated at the time of analysis. To determine IgG concentration, two unheparinized blood samples were also collected from the calves on day 50 and 60 after treatment. These blood samples were centrifuged (500 g for 15 min at 4°C), and the separated serum samples stored at -20˚C.

Analysis of chemical composition of the feed

During the experiment, feed samples of milk and starter were collected twice and dried at 55ºC. The starter was ground through a 1mm screen. Dry matter content was determined at 100ºC (ID 934.01; 22 AOAC, 2000). Nitrogen content was determined by the Kjeldahl method using automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). Acid and neutral detergent fiber content was sequentially determined using a Fiber Analyzer (Fiber Tic System, Tecator, 1010, Denmark) following the manufacturer's instructions, which were based on the methods described by Van Soest *et al*. (1991). Fat content was determined by extraction with ether using Soxlec System HT apparatus. Feed samples were analyzed for Ca, Zn, Mg, Mn, Fe and Cu by atomic absorption spectrophotometry (AAS: Perkin-Elmer 1981a), for P by colorimetry (AOAC 2000), sulfur by ICP (University of Tehran, the chemistry laboratory; Perkin-Elmer, 1981b), and Se by hydride generation AAS (University of Tehran, the chemistry laboratory; Perkin-Elmer 1981b), (Table 1).

Assay of blood parameters

Plasma concentration of cortisol was determined using radioimmunoassay kits (Coat-a-Count®; Diagnostic Products, Immuno Tech, Beckman Coulter Co, Czech Republic). The sensitivity and intra-assay coefficients of variation of the cortisol assay were 3.6 ng/ml and 2%, respectively. Red blood cell glutathione peroxidase activity (RBC GSH-PX-1), as enzyme unit per mg of hemoglobin was assayed using the method described by Paglia and Valentine (1967). Hemoglobin concentration was determined using the Cyanmethemoglobin method (Sigma, procedure No. 5250). Serum concentrations of IgG were measured by radial immunodiffusion (VMRD Inc, Pullman, WA).

Statistical Analyses

Plasma concentrations of cortisol and the body weights were analyzed according to a completely randomized design with repeated measurements using Proc MIXED of SASI (1990). The model included treatment (whether Sel-Plex was used), calves within the treatment, time of measurement and the interaction between treatment and time. The dependent variables were cortisol and body weight. Calves within each treatment were used as error terms in analyzing data. The effect of the treatment on RBC GSH-PX-1 and IgG concentration was analyzed using Proc GLM (SASI 1990) in a completely randomized design All results are presented as means ± the standard error of the mean (SEM), and differences between means were calculated for statistical significance, set at $p<0.05$.

Results

Intake and body weight changes

Intake of milk, starter and calf BW changes are shown in Tables 1 and 4. Environmental temperatures, humidity factors, and evaluated low critical temperatures are reported in Table 2. Feed conversion ratio and dry matter intake of milk and starter were not affected by inclusion of Sel-Plex into the diet. Although BW tended to increase ($p=0.09$) in the treated calves compared to control group, average daily gain was not affected by Sel-Plex supplementation. Time and primary body weight had a significant effect on BW ($p<0.01$). There was no significant effect of treatment × time on BW in the Sel-Plex group (Table 3). Examination during the experiment showed no toxicosis or signs of selenium poisoning. Although both groups were suffered by pneumonia, calves in Sel-Plex supplemented group showed less clinical intensity signs than control group.

Blood parameters

Calves fed additional Se did not show any significant difference in plasma cortisol concentration than calves in the control group (Tables 3 and 4) except in the third week of the experiment (Figure 1). Serum IgG concentration was significantly higher ($p<0.05$) in calves given Sel-Plex (Table 4). The glutathione peroxidase activity of calves was significantly greater in the Sel-Plex group ($p<0.01$) than that in the control group (Table 4).

Table 1: Composition and chemical analysis of feed (based on DM)

Ingredients of starter	Percent
Alfalfa	25
Barley	6.75
Corn	40.27
Wheat	2.11
Cotton seed meal	2.2
Soybean	10.32
Sugar beat	1.5
Mineral and vitamins premix[1]	0.75
Salt	0.225
Calcium carbonate	1.46
Zeolite	2.2
Monodicalcium phosphate	0.255
Sodium bicarbonate	0.36
Molasses-beet	2.25
Fish meal	2.85
Corn gluten meal	1.5

Chemical composition	Milk	Starter
Ash (% of DM)	5	10.18
EE[2] (% of DM)	28.46	3
NDF (% of DM)	-	12.8
ADF (% of DM)	-	11.6
CP (% of DM)	23.19	22
Lactose (% of DM)	43.35	-
Calcium (% of DM)	1.07	1.35
Phosphorus (% of DM)	0.71	0.32
Magnesium (% of DM)	0.07	0.35
Manganese (mg/kg)	0.2	16.95
Zinc (mg/kg)	8.38	12.27
Iron (mg/kg)	-	416.9
Copper mg/kg	0.3	13.36
Sulfur (sulfate, % of DM)	0.7	0.2
Selenium (mg/kg)	0.01	0.02
ME (Mcal/kg DM)	5.29	3.28
NEm (Mcal/kg DM)	3.34	2.27
NEg (Mcal/kg DM)	2.16	1.58

Mineral and vitamins premix contained no added Se. Each kg of this premix contained 500,000 IU vit A, 100,000 IU D_3, 100 IU vit E, 180,000 mg/kg Calcium, 90,000 mg/kg Phosphorus, 20,000 mg/kg Magnesium, 300 mg/kg Copper, 60,000 mg/kg Sodium, 3,000 mg/kg Iron, 2,000 mg/kg Manganese, 3,000 mg/kg Zinc, 100 mg/kg Cobalt, 100 mg/kg Iodine and 400 mg/kg Antioxidant. [2] Ether extract.

Table 2: Environmental temperature and humidity factors, and evaluated low critical temperature in Holstein calves

	Week							
Environmental factors	1	2	3	4	5	6	7	8
Moment temperature at blood sampling time, °C	14	15	2	13	13	14	14	22
Relative humidity at blood sampling time, %	75	73	72	60	57	63	72	59
Least temperature on blood sampling day, °C	---	6	-4	2.4	4	8.2	9.6	14
Mean weekly temperature, °C	---	9.2	9.7	11.6	11.2	12.5	14.6	17.9
Low critical temperature[1], °C	6.4	5.3	4.5	3.9	3.5	3.4	3.5	3.8

[1] Low critical temperature was evaluated according to age of calves (Gonzalez-Jimenez and Blaxter, 1962)

Table 3. Analysis of Sel-Plex effect on the body weight and plasma cortisol in the calves.

Variable	Effect of primary body weight or primary hormones	Treatment effect	Time effect	Treatment× time effect
Body weight (kg)	**	NS	**	NS
Cortisol (ng/ml)	NS	NS	*	NS

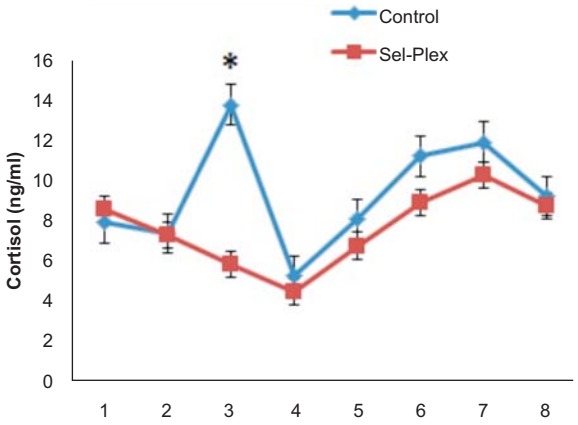
NS: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$.

Table 4. Effect of Sel-Plex on parameters in pneumonic Holstein suckling calves[1]

Parameters	Treatments		SEM	p-value
	Control	Sel-Plex		
Body weight (kg)	78.95	81.06	2.03	0.09
ADG (g/d)	1018	1065	82.75	0.7
DMI (milk and starter, kg/animal)	118.74	116.70	10.65	0.9
DMM[2] (kg/animal)	50.69	50.87	4.38	0.98
DMS[3] (kg/animal)	59.68	65.88	7.36	0.58
FCR[4] (DMI/weight gain)	1.92	1.94	0.05	0.8
GSH-PX-1 (EU/gHb)[5]	19.39	40.70	3.96	0.01
IgG (mg/ml)	32.21	44.37	3.1	0.04
Cortisol (ng/ml)	9.23	7.51	1.76	0.17

[1]Selenium drench milk (0.3 mg/kg Sel-Plex per kg in dry matter intake of milk) or unsupplemented milk (milk without Sel-Plex supplementation) was administered daily to treatment and control (n = 5) calves, beginning February 4, 2006, and ending April 4, 2006; five calves were bled for each group during the experimental period (60 day); Data are least squares means. [2]Total dry matter milk. [3]Total dry matter starter. [4]Feed conversion ratio. [5]Erythrocyte glutathione peroxidase activity (RBC GSH-PX-1) expressed as enzyme units per gram of hemoglobin (EU/g of hemoglobin). One enzyme unit is the activity needed to oxidize 1 mol of NADPH/min.

Figure 1. The effects of Sel-Plex on calf plasma cortisol (mean ± SEM), (*$p < 0.05$).



Discussion

Previous studies on the impact of Sel-Plex supplementation BW have produced mixed results. Some studies (Swecker *et al*., 1989; Lacetera *et al*., 1996; Bruce, 1997; Awadeh *et al*., 1998; Gunter *et al*., 2003; Chung *et al*., 2007; Lee *et al*., 2007) have reported no significant impact of Se supplementation on BW gain of cows and their calves. In contrast, others (Gleed *et al*., 1983; Spears *et al*., 1986; Wichtel *et al*., 1996; Yue *et al*., 2009) have reported positive effects of Se supplementation on weight gain and/or average daily gain in young cattle. Evidence suggests a role for Se in growth, since Se deficiency can reduce pituitary concentration of GH by impairing the production of triiodothyronine (T_3), (Arthur *et al*., 1990). Peripheral GH concentration did not change in Se deficiency, suggesting that Se could alter somatotropic function

via the endocrine or paracrine production of IGF-I, secretion of IGF-II, the number of somatotropic receptors, or the peripheral concentration of IGF binding proteins (Arthur *et al*., 1990; Wichtel *et al*., 1996). Therefore, the non-significant but positive effect of Sel-Plex on the BW of calves with marginal selenium status may be mediated by increased activity of type II 5′-deiodinase and greater synthesis of IGF-I. The minimal effect of Sel-Plex on dry matter intake of milk and starter is consistent with other studies (Mahan *et al*., 1999; Rock *et al*., 2001). Rock et al. (2001) reported that neither source nor level of supplemented Se affected intake of the basal diet of ewes. Similarly, Mahan and Parret (1996) and Mahan *et al*. (1999) demonstrated that neither source nor level of Se had an effect on pig weight gain, feed intake, or gain: feed ratio compared with the basal diet. Although Sel-Plex had no significant effect on plasma cortisol concentration, there was a tendency to lower cortisol concentration. Our results were consistent to Gupta *et al*. (2005) who reported significantly lower cortisol concentrations at parturition in cows supplemented with Se. Our findings therefore suggest that Se did not significantly affect stress in calves. There was a significant difference in plasma cortisol concentration between the two groups in the third week, when the environmental temperature was 2°C (Table 2). Since this is below the low critical temperature for calves (Davis and Drackley, 1998), activity in the hypothalamus-pituitary-adrenal axis may increase, leading to a greater concentration of plasma cortisol in the control group (Balm, 1999). In the treatment group, an increase in thyroid hormone activity which is resulted by adding selenium supplementation, and consequently more heat production, may reduce the effect of cold stress (Ebrahimi *et al*., 2009) and therefore limit the increase in cortisol concentration. The increased activity of glutathione peroxidase in calves fed Sel-Plex is in agreement with other studies (Backall and Scholz, 1981; Stowe and Herdt, 1992; Gunter *et al*., 2003; Rowntree *et al*., 2004; Yue *et al*., 2009). Additional Se fed to suckling calves leads to an increase in glutathione peroxidase activity in a shorter time than in mature cows. However, because the duration of our experiment was shorter than the red blood cell lifespan, the maximum activity of GSH-PX-1 may not have been recorded, and consequently we simply reported the amount of RBC GSH-PX-1 activity at the end of the experiment. Increasing serum concentration of IgG with pneumonia was in agreement with other studies (Afzal *et al*., 1988; Larsen, 1993; Swecker *et al*., 1995; Lacetera *et al*., 1996; Awadeh *et al*., 1998; Rock *et al*., 2001; Mudgal, 2005; Shinde *et al*., 2007). These studies indicate that Se supplementation during the late gestation can affect both the maternal synthesis of IgM and IgG and the absorption of IgG by newborn calves. In the current study, we administered Se directly in the milk of suckling calves and observed a significant increase in IgG concentration. Since the calves were suffering from pneumonia during the experiment, this condition led to greater antibody production against infection in the Sel-Plex group in comparison with the control group. Therefore, it seems that Sel-Plex enhances recovery from pneumonia in calves. Accordingly, Suckling calves which their dams were fed extensively on Se-deficient feedstuffs may be at risk of Se deficiency (Pehrson *et al*., 1999). However, the results of present study indicated that Sel-Plex supplementation in milk of suckling calves with a marginal selenium status increased the whole blood glutathione peroxidase activity (which shows increase in selenium content of body) and serum concentration of IgG, and tended to increase body weight.

References

1. Afzal, M.; Hussain, M.; Khan, K.N.M. and Munir, R. (1988) Effect of vitamin E and selenium on immunity in newborn Jersey and Buffalo calves. Asian-Aust. J. Anim. Sci. 1: 13-19.
2. AOAC (2000) Official methods of analysis. Association of Official Analytical Chemists. EUA.
3. Arthur, J.R.; Nicol, F.; Rae, P.W.H. and Beckett, G.J. (1990) Effects of selenium deficiency on the thyroid gland and on plasma and pituitary thyrotropin and growth hormone concentrations in the rat. Clin. Chem. Enzyme Commun. 3: 209.
4. Awadeh, F.T.; Kincaid, R.L. and Jhonson, K.A. (1998) Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. J. Anim. Sci. 76: 1204-1215.
5. Backall, K.A.; Scholz, R.W. (1981) Reference values for a field test to estimate inadequate glutathione peroxidase activity and selenium status in the blood of cattle. Am. J. Vet. Res. 40: 733-738.
6. Balm, H.M. (1999) Stress physiology in animal. CRC Press. LLC. pp: 15-30.
7. Bruce, L.B. (1997) Effects of selenium on cold adapted beef cattle. Asian-Aust. J. Anim. Sci. 11: 265-567.
8. Chung, J.Y.; Kim, J.H.; Ko, Y.H. and Jang, I.S. (2007) Effects of dietary supplemented inorganic and organic selenium on antioxidant defense systems in the intestine, serum, liver and muscle of Korean native goats. Asian-Aust. J. Anim. Sci. 20: 52-59.
9. Davis, C.L.; Drackley, J.K. (1998) The development, nutrition, and management of the young calves. Iowa State. University Press. Ames. IA.
10. Ebrahimi, M.; Towhidi, A. and Nikkhah, A. (2009) Effect of organic selenium (Sel-Plex) on thermometabolism, blood chemical composition and weight gain in Holstein suckling calves. Asian-Aust. J. Anim. Sci. 22: 984-992.
11. Gleed, P.T.; Allen, W.M.; Mallinson, C.B.; Rowlands, G.J.; Sansom, B.F.; Vagg, M.J. and Caswell, R.D.

(1983) Effects of selenium and copper supplementation on the growth of beef steers. Vet. Rec. 113: 388-392.
12. Gonzalez-Jimenez, E.; Blaxter K. L. (1962) The metabolism and thermal regulation of calves in the first month of life. Br. J. Nutr. 16: 199-212.
13. Gunter, S.A.; Beck, P.A.; Phillips, J. M., 2003: Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. J. Anim. Sci. 81, 856-864.
14. Gupta, S.; Gupta, H.K.; Soni, J. (2005) Effect of vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retained fetal membranes in crossbred dairy cattle. Theriogenology. 64: 1273-1286.
15. Hoekstra, W.G. (1974) Biochemical Role of Selenium. Pages 61-77 in Trace Element Metabolism in Animals, Vol. II. (Ed. Hoekstra, W. G.; Suttie, J. W.; Ganther, H. E.; Mertz, W.). Univ. Park Press, Baltimore, MD.
16. Kiremidjian, L. K.; Stotzky, G. (1987) Selenium and immune responses. Environ. Res. 42: 277-303.
17. Lacetera, N.; Bernabuci, U.; Ronchi, B. and Nardone, A. (1996) Effects of selenium and vitamin E administration during a late stage of pregnancy on colostrums and milk production in dairy cows, and on passive immunity and growth of their offspring. Am. J. Vet. Res. 57: 1776-1780.
18. Larsen, H. J. (1993) Relation between selenium and immunity. Norw. J. Agric. Sci. Suppl. 11: 105-119.
19. Lee, S.H.; Park, B.Y.; Yeo, J.M.; Lee, S.S.; Lee, J.H.; Ha, J.K. and Kim, W.Y. (2007) Effects of different selenium sources on performance, carcass characteristics, plasma glutathione peroxidase activity and selenium deposition in finishing Hanwoo steers. Asian-Aust. J. Anim. Sci. 20, 229-236.
20. Mahan, D. C.; Cline, T. R. and Richert, B. (1999) Effects of dietary levels of selenium-enriched yeast and sodium selenite as selenium sources fed to growing-finishing pigs on performance, tissue selenium, serum glutathione peroxidase activity, carcass characteristics, and loin quality J. Anim. Sci. 77: 2172-2179.
21. Mahan, D.C.; Parrett, N.A. (1996) Evaluating the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine. J. Anim. Sci. 74: 2967-2974.
22. Mudgal, V. (2005) Effect of dietary supplementation of copper, selenium and zinc on growth performance, nutrient utilization, antioxidant status and immune response in male buffalo calves. (Unpublished PhD Thesis, Deemed University, Indian Veterinary Research Institute, Izatnagar, India.)
23. NRC. (2001) Nutrient requirements of the young calf. 7th Rev. Ed. Natl. Acad. Press, Washington, DC. pp: 214-233.
24. Paglia, D. E.; Valentine, W. N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70:158-169.
25. Pehrson, B.; Ortman, K.; Madjid, N. and Trafikowsk, U. (1999) The influence of dietary selenium as selenium yeast or sodium selenite on the concentration of selenium in the milk of suckler cows and on the selenium status of their calves. J. Anim. Sci. 77: 3371-3376.
26. Perkin-Elmer. (1981a) Analytical methods for atomic absorption spectroscopy. Perkin-Elmer Tech. pp: 39.
27. Perkin-Elmer. (1981b) Analytical methods for atomic absorption spectroscopy using the MHS mercury hydride system. Publication No. 309. pp: 13.
28. Rock, M.J.; Kincaid, R.L. and Carstens, G.E. (2001) Effect of prenatal source and level of dietary selenium on passive immunity and thermometabolism of newborn lambs. Small Rumin. Res. 40: 129-138.
29. Rowntree, J.E.; Hill, G.M.; Hawkins, D.R.; Link, J.E.; Rincker, M.J.; Bednar, G.W. and Kreft, R.A. (2004) Effect of Se on selenoprotein activity and thyroid hormone metabolism in beef and dairy cows and calves. J. Anim. Sci. 82: 2995-3005.
30. SAS1. (1990) User's Guide, Statistics, Version 6 Edition. SAS Institute, Inc., Cary, NC.
31. Shinde, P.L.; Dass, R.S.; Garg, A.K. and Chaturvedi, V.K. (2007) Immune Response and Plasma Alpha Tocopherol and Selenium Status of Male Buffalo (*Bubalus bubalis*) Calves Supplemented with Vitamin E and Selenium. Asian-Aust. J. Anim. Sci. 20: 15391545.
32. Spears, J.W.; Harvey, R.W. and Segerson, E.C. (1986) Effects of marginal deficiency and winter protein supplementation on growth, reproduction, and selenium status of beef cattle. J. Anim. Sci. 63: 586-594.
33. Stowe, H.D.; Herdt, T.H. (1992) Clinical assessment of selenium status of livestock. J. Anim. Sci. 70: 3928-3933.
34. Swecker, W.S.; Eversole, D.E.; Thatcher, C.D.; Blodgett, D.J.; Schurig, G.G. and Meldrum, J.B. (1989) Influence of supplemental selenium on humoral immune responses in weaned beef calves. Am. J. Vet. Res. 50: 1760-1763.
35. Swecker, W.S.; Thatcher, C.D.; Eversole, D.E.; Blodgett, D.J. and Schurig, G.G. (1995) Effect of selenium supplementation on colostral IgG concentrations in cows grazing selenium-deficient pastures and on post suckle serum IgG concentration in the calves. Am. J. Vet. Res. 56: 450-453.
36. Underwood, E.J. (1977) Trace elements in human and animal nutrition. 4th Edition. Acad. Press, Inc., New York, NY.
37. Van Soest, P.J.; Robertson, J.B. and Lewis, B.A. (1991) Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.
38. Weiss, W.P. (2003) Selenium nutrition of dairy cows: comparing responses to organic and inorganic selenium forms. In Proceeding of the 19th Alltech Annual Symposium Nutrition, Biotechnology Feed Food: 20–23; Lexington. Nottingham University Press, Nottingham, UK; 2003:333-373.

39. Wichtel, J.J.; Craigie, A.L.; Freeman, D.A.; Alvarez, H.V. and Williamson, N.B. (1996) Effect of selenium and iodine supplementation on growth rate and on thyroid and somatotropic function in dairy calves at pasture. J. Dairy Sci. 79: 1866-1872.
40. Yue, W.; Zhang, C.; Shi, L.; Ren, Y.; Jiang, Y. and Kleemann, D.O. (2009) Effect of supplemental selenomethionine on growth performance and serum antioxidant status in Taihang Black goats. Asian-Aust. J. Anim. Sci. 22: 365-370.