



## Effect of Organic Selenium (Sel-Plex) on Thermometabolism, Blood Chemical Composition and Weight Gain in Holstein Suckling Calves

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**ABSTRACT :** The objective of this study was to determine the effects of milk supplemented with Sel-Plex on thyroid hormones, rectal temperature, plasma glucose and cholesterol concentrations, and body weight in suckling calves. Ten Holstein suckling male calves of approximately 1 month of age were selected and randomly allocated to one of two groups and fed either unsupplemented milk (control) or milk supplemented with 0.3 mg/kg DM Se from Sel-Plex (treated). The animals received treated milk and free choice starter for two months. Dry matter intake for each individual animal was recorded daily throughout the study. The calves were weighed and blood samples were taken weekly. Plasma concentrations of triiodothyronine ( $T_3$ ), thyroxin ( $T_4$ ) and  $T_3$ -Uptake were determined using radioimmunoassay kits. Plasma cholesterol and glucose concentrations were determined by enzymatic-colorimetric methods. Rectal temperature was recorded at blood sampling time. During the experiment, ambient temperature and relative humidity were recorded at the time of blood sampling and every four hours. Treatment significantly ( $p < 0.01$ ) affected the concentration of  $T_3$ , free  $T_3$  index ( $FT_3I$ ) and ratio of  $T_3:T_4$  in plasma. The plasma concentrations of  $T_3$  in treated calves were 33% higher than in the control. The plasma concentrations of  $T_4$  ( $p < 0.05$ ), glucose ( $p < 0.01$ ) and cholesterol ( $p < 0.01$ ) were decreased in the treated group. Calves fed Sel-Plex had higher rectal temperature ( $p < 0.01$ ). Plasma free  $T_4$  index ( $FT_4I$ ) did not differ significantly between the two groups. An increase in body weight was observed ( $p < 0.09$ ) in the treated group. The results indicated that milk fortified with Sel-Plex could increase tissue conversion of  $T_4$  to  $T_3$  and therefore improve thermometabolism in suckling calves. (**Key Words :** Thermometabolism, Blood Chemical Composition, Weight Gain, Calf, Selenium)

### INTRODUCTION

Nutritional degenerative myopathy (white muscle disease) and poor growth rate of calves are common manifestations of Se deficiency (Underwood, 1977). Thyroid hormones are important for metabolism, development, and regulation of heat production. During Se deficiency some key selenoproteins including type I and type II-deiodinase are decreased. These enzymes are responsible for catalyzing the prohormone thyroxin ( $T_4$ ) to the more biologically active thyroid hormone 3, 5, 3 triiodothyronine ( $T_3$ ), (Croteau et al., 1996). Consequently, at cold stress which body needs more deiodinases and active thyroid hormones, more selenium is needed.

As an integral part of the enzyme glutathione peroxidase (GSH-Px), Se functions to prevent oxidative damage to body tissues (Hoekstra, 1974). In cold stress, there is an

increase in producing peroxides in most tissues, therefore more glutathione peroxidase activity and more selenium are needed. Currie (1995) reported that young calves were susceptible to cold stress in winter. That whole blood selenium concentration had been higher when organic selenium was fed compared with selenite (weiss, 2005), hence, we preferred to use Sel-Plex as organic selenium to any other forms of selenium. Because in most studies (Awadeh et al., 1998) prenatal supplementation of selenium was used to protect calves from selenium deficiency, in our study we directly supplemented calves with Sel-Plex. Accordingly, the objectives of this study were to determine the effect of supplemented milk with Sel-Plex on thyroid hormones, rectal temperature, blood chemical concentrations, and body weight in Holstein suckling calves.

### MATERIALS AND METHODS

#### Animals and location

On February 4, 2006, ten Holstein male calves with

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approximately one month of age were selected from the dairy herd of the department of Animal Science, University of Tehran, Karaj (35°48'N, 51°2'E). The suckling calves (average BW 47.15±4.1 kg) were randomly assigned to two groups (n = 5). They were maintained in individual calf pens inside a building.

### Experimental design

Calves had free choice starter with: 1- unsupplemented milk 2- milk supplemented with 0.3 mg/kg Se (dry matter intake of milk) as Sel-Plex for two months during winter. Sel-Plex (Alltech, USA) contained 500 g/kg CP, 3.7 g/kg CF, 50 g/kg ADF, 58 g/kg ASH, 1.1 mg/kg Se. Milk intakes were set at 10% of BW and were adjusted based on BW weekly, dry matter intake was calculated and in the milk of suckling calves 0.3 mg Se from Sel-Plex per kg of dry matter intake of milk was added (according to Alltech's recommendation). All calves had ten days adaptation before starting the experiment. Calves had *ad libitum* access to water. Starter was formulated to meet animal requirements for energy, protein, vitamins, and minerals (except Se) for calves (NRC, 2001), (Table 1). Dry matter intake of starter and milk were recorded daily for each calf during the experiment. Rectal temperatures were measured at the time of blood sampling (at 9:00 am). Ambient temperature and relative humidity were recorded at the time of blood sampling and every four hours inside. Body weight was registered weekly after feeding and watering restriction for 12 h at 9:00 am. Blood samples were also collected from all calves weekly at 9:00 am before feeding 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 obtained from the calves through the jugular vein by heparinized tubes and kept at 4°C. The samples were centrifuged (3,000×g for 20 min at 4°C), and plasma were separated and stored at -20°C until hormones analyses. At the end of trial, blood samples were also obtained, and heparinized whole blood samples were stored at -80°C for determining glutathione peroxidase activity.

### Chemical composition of feed

Feed samples (milk and starter) were collected twice during the trial, dried at 55°C. The starter was ground through a 1mm screen. Dry matter content was determined at 100°C (ID 934.01; 22 AOAC, 2000). Content of N in the samples were determined by Kjeldahl method in an automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). Acid detergent fiber and neutral detergent fiber were sequentially determined using a Fiber Analyzer (Fiber

**Table 1.** Composition of free choice starter and chemical analysis of feed were offered to male calves (based on dry matter)

Ingredients of starter	Percent	
Hay	25	
Barely	6.75	
Corn	40.27	
Wheat	2.11	
Cotton seed meal	2.2	
Soybean	10.32	
Sugar beat	1.5	
Mineral and vitamins premix <sup>1</sup>	0.75	
Salt	0.225	
Calcium carbonate	1.46	
Zeolite	2.2	
Monocalcium 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 <sup>2</sup> (% 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

<sup>1</sup> Mineral and vitamins premix contained no added Se. Each kg of this premix contained 500,000 IU vit A, 100,000 IU D<sub>3</sub>, 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.

<sup>2</sup> Ether extract.

tic system, Tecator, 1010, denmark) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Fat was determined by extraction with ether using a Soxhlet system HT apparatus. Feed samples were analyzed for Ca, Zn, Mg, Mn, Fe and Cu by atomic absorption spectrophotometry (AAS: Perkin-Elmer, 1981a). P by colorimetry (AOAC,

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

Environmental factors	Week							
	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 <sup>1</sup> (°C)	6.4	5.3	4.5	3.9	3.5	3.4	3.5	3.8

<sup>1</sup> Low critical temperature was evaluated according to age of calves (Gonzalez-Jimenez and Blaxter, 1962).

2000), selenium and sulfur by ICP method (Perkin-Elmer, 1981b), (Table 1).

#### Assay of hormones and glutathione peroxidase

Total concentrations of T<sub>3</sub> and T<sub>4</sub> were determined, using radioimmunoassay kits (Coat-a-Count<sup>®</sup>; Diagnostic Products, Kavoshyar, Co, Iran). Sensitivity and intraassay coefficients of variation of the T<sub>3</sub> assay were 0.1 nmol/L and 4.2%, respectively. Sensitivity and intra-assay coefficients of variation of the T<sub>4</sub> assay were 0.2 nmol/L and 7.9%, respectively. The percent of T<sub>3</sub>-Uptake was determined using a double-antibody radioimmunoassay kits (Coat-a-Count<sup>®</sup>; Diagnostic Products, Bouty Italy, CO). Sensitivity and intra-assay coefficients of variation of the T<sub>3</sub>-Uptake assay were 0.1% and 3.5%, respectively. Then, free T<sub>3</sub> index (FT<sub>3</sub>I) and free T<sub>4</sub> index (FT<sub>4</sub>I) were calculated by using the following formulas: FT<sub>3</sub>I = T<sub>3</sub>×T<sub>3</sub>-Uptake and FT<sub>4</sub>I = T<sub>4</sub>×T<sub>3</sub>-Uptake.

Plasma cholesterol and glucose concentrations were determined by the Enzymatic, Colorimetric method. Plasma glucose and cholesterol reagents were supplied by Sigma Diagnostics (Zist Shimi Co), and spectrophotometric assays were performed.

Selenium-dependent glutathione peroxidase (GSH-PX-1) activity (as enzyme unit per milligram of hemoglobin) in blood was assayed with the method of Paglia and Valentine (1967). Hemoglobin was determined with the Cyanmethemoglobin method (Sigma, procedure No. 5250).

#### Statistical analyses

Plasma concentrations of T<sub>3</sub>, T<sub>4</sub>, FT<sub>3</sub>I, FT<sub>4</sub>I, cholesterol, glucose, and rectal temperature and weight were analyzed according to a completely randomized design with repeated measurements using Proc MIXED of SAS (1990). The model included treatment (Sel-Plex used in milk), calves within treatment, time of measure and the treatment×time interaction. Dependent variables were T<sub>3</sub>, T<sub>4</sub>, FT<sub>3</sub>I, FT<sub>4</sub>I, cholesterol and glucose, and rectal temperature and weight. Calves within treatment were used as error terms to test. The effect of treatment on glutathione peroxidase activity in whole blood was analyzed using Proc GLM in a completely

randomized design in SAS (1990). All results are presented as means±standard error of means (SEM). Differences between means was calculated for statistical significances (p<0.05).

Statistical model of Mixed Model is described below:

$$Y_{ij} = \mu + T_i + Z_j + TZ_{ij} + b_1(D) + b_2(C_0) + b_3(W_0) + e_{ijk}$$

Where Y<sub>ij</sub> was the dependent variable, μ is the overall mean; T<sub>i</sub> is the effect of the treatments (I = 1, 2), Z<sub>j</sub> is the time effect, TZ<sub>ij</sub> is the effect of treatment×time, D is the effect of Environmental temperature, C<sub>0</sub> is the effect of Primary concentration of hormones, W<sub>0</sub> is the effect of Primary body weight, b<sub>1</sub> is Linear regression index Y of environmental temperature, b<sub>2</sub> is Linear regression index Y of primary concentration of hormones, b<sub>3</sub> is Linear regression index Y of Primary body weight, and e<sub>ijk</sub> is the experimental error. Effects of the treatments were declared significant at p<0.05.

## RESULTS

#### Intake and body weight change

Intake of milk, starter and body weight change data are shown in Table 3 and 4. Dry matter intake of milk and starter were not affected with supplementation of Sel-plex. Feed conversion ratio was similar between treatments. Although body weight tended to increase (p = 0.09) in the treated calves compared to control, average daily gain was not affected by Sel-Plex supplementation. Time and primary body weight had a significant effect on body weight (p<0.01). There was no effect of treatment×time for body weight in Sel-Plex group than control group (Table 3).

#### Thyroid hormone concentrations

Sel-Plex supplementation to the milk of suckling calves with a marginal selenium status affected the concentration of T<sub>3</sub> (p<0.01). Mean plasma concentration of T<sub>3</sub> was 33% greater in Sel-Plex group than in control group (Table 4), (Figure 1A). There was an effect of treatment, time and treatment×time for T<sub>3</sub> in Sel-Plex group than control group,

**Table 3.** Analysis of Sel-Plex treatment, time and treatment×time effect on thyroid hormone concentrations, plasma glucose and cholesterol concentrations, rectal temperature, glutathione peroxidase activity and body weight in Holstein suckling calves

Variable	Effect of primary body weight or primary hormones	Treatment effect	Time effect	Treatment×time effect
Body weight (kg)	**	NS	**	NS
T <sub>3</sub> (nmol/L)	NS	**	**	**
T <sub>4</sub> (nmol/L)	NS	*	**	NS
T <sub>3</sub> /T <sub>4</sub> (%)	NS	**	**	**
FT <sub>3</sub> I (nmol/L)	NS	**	**	*
FT <sub>4</sub> I (nmol/L)	NS	NS	**	NS
Rectal temperature (°C)	NS	**	**	NS
Glucose (mg/dl)	NS	**	**	NS
Cholesterol (mg/dl)	NS	**	*	NS

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

**Table 4.** Effect of Sel-Plex during 60 days on thyroid hormone concentrations, plasma glucose and cholesterol concentrations, rectal temperature, glutathione peroxidase activity and body weight in Holstein suckling calves<sup>1</sup>

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 <sup>2</sup> (kg/animal)	50.69	50.87	4.38	0.98
DMS <sup>3</sup> (kg/animal)	59.68	65.88	7.36	0.58
FCR <sup>4</sup> (DMI/weight gain)	1.92	1.94	0.05	0.8
T <sub>3</sub> <sup>5</sup> (nmol/L)	1.94	2.58	0.1	0.01
T <sub>4</sub> <sup>6</sup> (nmol/L)	59.76	54.01	2.12	0.05
T <sub>3</sub> /T <sub>4</sub> (%)	3.46	4.79	0.18	0.01
FT <sub>3</sub> I <sup>7</sup> (nmol/L)	0.94	1.28	0.06	0.01
FT <sub>4</sub> I <sup>8</sup> (nmol/L)	29.5	27.58	1.5	0.23
Rectal temperature (°C)	38.89	39.04	0.57	0.01
Glucose (mg/dl)	55.60	49.60	0.9	0.01
Cholesterol (mg/dl)	115.93	105.89	2.6	0.01
GSH-PX (EU/gHb) <sup>9</sup>	19.39	40.70	3.96	0.01

<sup>1</sup> Selenium drench milk (0.3 mg/kg seleno-yeast per kg in dry matter intake of milk) or unsupplemented milk (milk without seleno-yeast 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.

<sup>2</sup> Total dry matter milk. <sup>3</sup> Total dry matter starter. <sup>4</sup> Feed conversion ratio. <sup>5</sup> T<sub>3</sub> = triiodothyronine.

<sup>6</sup> T<sub>4</sub> = thyroxine. <sup>7</sup> FT<sub>3</sub>I = Free T<sub>3</sub> index. <sup>8</sup> FT<sub>4</sub>I = Free T<sub>4</sub> index.

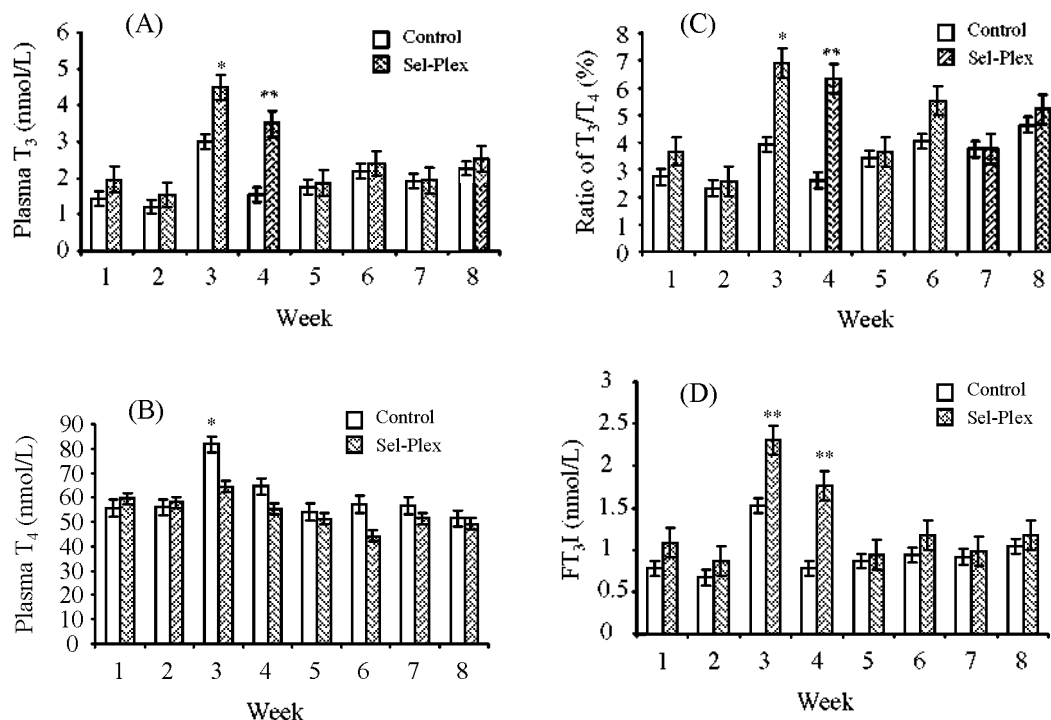
<sup>9</sup> Erythrocyte glutathione peroxidase activity (RBC GSH-PX-1) expressed as enzyme units per gram of hemoglobin (EU/g of hemoglobin). One enzyme unit is the activity needed to oxidize 1 mol of NADPH/min.

but there was no effect of primary T<sub>3</sub> concentration (Table 3).

Treatment also affected concentration of T<sub>4</sub> (p<0.05) with 11% lower in Sel-Plex group than in control group (Figure 1B). There was an effect of treatment and time for T<sub>4</sub> in Sel-Plex group than in control group, but there was no effect of treatment×time and primary T<sub>4</sub> concentration (Table 3).

The T<sub>3</sub> to T<sub>4</sub> ratios significantly (p<0.01) increased in Sel-Plex group than in control (Figure 1C). There was an effect of treatment, time and treatment×time for T<sub>3</sub> to T<sub>4</sub> ratios in Sel-Plex group than in control group, but there was no effect of primary T<sub>3</sub> to T<sub>4</sub> ratios (Tables 3 and 4).

FT<sub>3</sub>I (an index of thermometabolism) was affected by Sel-Plex treatment and it was 36% greater in Sel-Plex group than in control group (Figure 1D), however FT<sub>4</sub>I was not



**Figure 1.** The effects of Sel-Plex on plasma concentrations (Mean±SEM) of 3, 5, 3'-triiodo-L-thyronine (T<sub>3</sub>) (A), L-thyroxin (T<sub>4</sub>) (B), ratio of T<sub>3</sub>/T<sub>4</sub> (C) concentrations and Free T<sub>3</sub> index (FT<sub>3</sub>I) (D) in Holstein suckling calves (n = 5 per each group). Hormones concentrations and rectal temperature were compared weekly (significant differences between treatment and control on each week) during the experiment (\* p<0.05, \*\* p<0.01).

affected by treatments. There was an effect of treatment, time and treatment×time for FT<sub>3</sub>I in Sel-Plex group than in control group, but there was no effect of primary FT<sub>3</sub>I concentration (Tables 3 and 4).

#### Rectal temperature

The rectal temperature of calves with a marginal selenium status was affected by Sel-Plex treatment (p<0.01) and it was greater in Sel-Plex group than in control (Table 4), (Figure 2A). There was an effect of treatment and time for rectal temperature in Sel-Plex group than in control group, but there was no effect of treatment×time (Table 3).

#### Plasma chemical composition and glutathione peroxidase enzyme

Sel-Plex supplementation reduced plasma glucose concentrations (p<0.01) of calves with a marginal selenium status (Table 4), (Figure 2B). There was an effect of treatment and time for glucose in Sel-Plex group than in control group, but there was no effect of treatment×time and primary glucose concentration (Table 3).

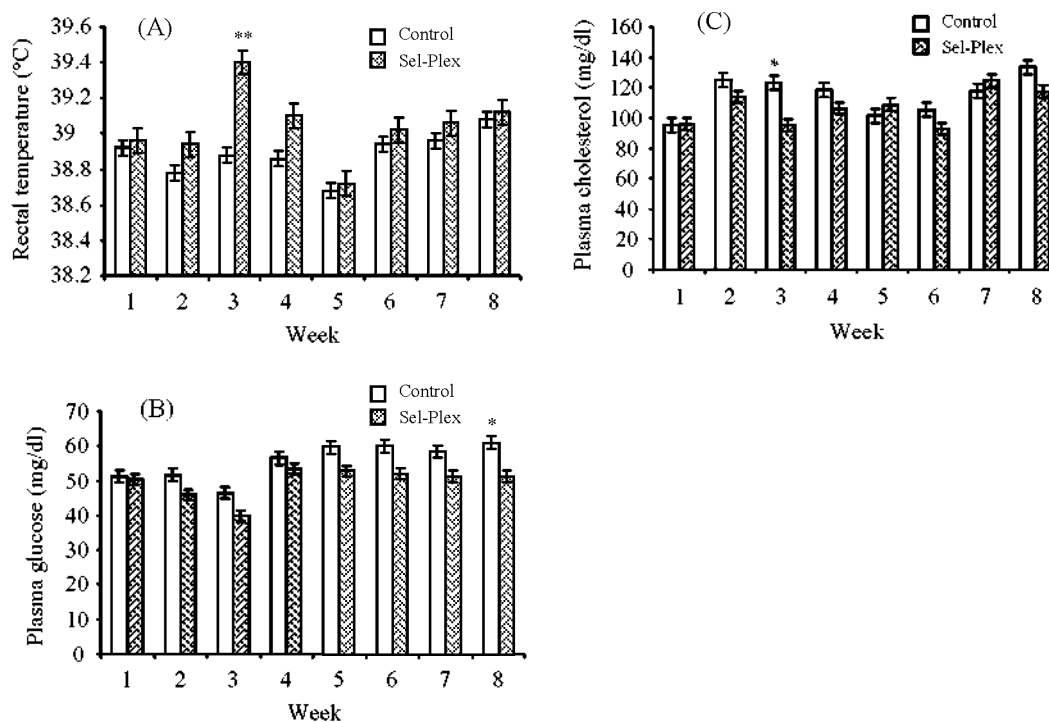
Plasma cholesterol concentration was affected by Sel-Plex treatment (p<0.01) and it was lower in Sel-Plex group than in control group (Table 4), (Figure 2C). There was an effect of treatment and time for cholesterol in Sel-Plex group than control group, but there was no effect of

treatment×time and primary cholesterol concentration (Table 3).

In the present study, glutathione peroxidase activity of calves was affected by treatment (p<0.01) and it was 110% greater in Sel-Plex group than control group (Table 4).

#### DISCUSSION

Studies on the impact of Sel-Plex supplementation on body weight change have also produced mixed results. Research in Alaska showed that cows administered an intraruminal selenium bolus (sodium selenite) did not differ in BW from cows not given a Se bolus (Bruce, 1997). Chung et al. (2007) reported that organic and inorganic selenium had not any significant effect on body weight and total gain of Korean native goats. In finishing Hanwoo steers, selenium supplementation had not any significant effect on dry matter intake and body weight gain (Lee et al., 2007). Other studies (Swecker et al., 1989; Lacetera et al., 1996; Awadeh et al., 1998; Gunter et al., 2003) have also reported no significant impact of Se supplementation on body weight gain of cows and on weight gain of their calves. In contrast, others (Gleed et al., 1983; Spears et al., 1986; Wichtel et al., 1996; Yue et al., 2009) reported positive effects of selenium supplementation on weight gain and/or ADG in young cattle. Spears et al. (1986) reported cows consuming diets



**Figure 2.** The effects of Sel-Plex supplementation on rectal temperature (A), plasma concentrations of glucose (B) and cholesterol (C) (Mean $\pm$ SEM) of suckling calves compare with unsupplemented group during the experiment. Significant differences between treatment and control on each week were reported (\*  $p < 0.05$ , \*\*  $p < 0.01$ ).

that were moderately deficient in selenium (0.05 to reported 0.09 mg/kg) and were injected monthly with sodium selenite and vitamin E lost less BW during the winter than non-injected cows. However, the injected cows gained less BW during the summer than non-injected cows. However, in all of these studies, the basal diet of the animals was extremely deficient in Se, and no effect of Se supplementation on weight gain has been found when the basal diet fed to young cattle was marginal or normal in Se content. Evidence exists that selenium deficiency can reduce pituitary concentration of GH by impairing production of  $T_3$  by type II 5'-deiodinase in the pituitary and suggested a role for Se in growth (Arthur et al., 1990). Peripheral GH did not change in selenium deficiency, suggesting that selenium could alter somatotrophic function through the effects on the endocrine or paracrine production of IGF-I, secretion of IGF-II, the number of somatotrophic receptors, or the peripheral concentration of IGF binding proteins (Arthur et al., 1990; Wichtel et al., 1996). Therefore slight insignificant positive effect of Sel-Plex on body weight of Holstein suckling calves with marginal selenium status may be mediated by more activity of type II 5'-deiodinase and more synthesis of IGF-I.

The lack of dry matter intake of milk and starter to Sel-Plex is in agreement 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. Mahan and Parret. (1996) and Mahan et al. (1999) demonstrated that neither Se source nor dietary Se level had any effect on pig gain, feed intake, or gain:feed ratio compared with the non-Se-fortified basal diet.

Weekly comparison of the Sel-Plex treatment with the control group showed that there was a significant increase in the amount of  $T_3$  concentrations in the third and fourth weeks of experiment (Figure 1A). Also weekly comparison of the Sel-Plex treatment with the control group showed that there was a significant decrease in the amount of  $T_4$  concentrations in the third week of experiment (Figure 1B) and there was a significant increase in the percentage of  $T_3$  to  $T_4$  ratios (Figure 1C) and the  $FT_3I$  (Figure 1D) in the third and fourth weeks of experiment. This result is in agreement with other studies (Awadeh et al., 1998; Beckett et al., 1993; Donald et al., 1994; Cammack et al., 1995; Thompson et al., 1995). In newborn lambs (Donald et al., 1994) and calves (Awadeh et al., 1998) whose dams supplemented with selenium, and growing male calves (Arthur et al., 1988) and heifers (Wichtel et al., 1996), selenium supplementation increased plasma concentrations of  $T_3$  and decreased plasma concentrations of  $T_4$ . Lower  $T_3$  to  $T_4$  ratios in unsupplemented groups in those studies showed that 5'-deiodinase activity was impaired by selenium deficiency. Whereas, Rock et al. (2001) reported that dietary selenium in pregnant ewes increased the  $T_3$  and  $T_4$  concentration in plasma of ewes, but it had no effect on

ratios of  $T_3$  to  $T_4$ , and also selenium treatment did not affect thyroid hormones in newborn lambs. Conversion of  $T_4$  to  $T_3$  is controlled by three isoenzymes: type 1 (IDI), (in liver, kidney, and thyroid), type 2 (IDII), (in brain, brown adipose tissue, and pituitary), and type 3 (IDIII), (in brain and placenta). About 80% of  $T_3$  in plasma is produced in the liver, kidney, muscle, and all these tissues contain the selenium-dependent enzyme type IDI (Beckett et al., 1992). In rats, Se deficiency has been shown to reduce the activity of 5'-deiodinase in liver (Beckett et al., 1992).

In the current study, in the third week of experiment, the moment temperature at the time of blood sampling and also the least temperature on the day of blood sampling were 2 and -4°C respectively, well below temperature reported to induce cold stress (low critical temperature) in the calves of this age (Table 2); and therefore calves were under environmental cold stress. Because environmental temperatures below the low critical temperature lead to cold stress for animals (Davis and Drackley, 1998), this condition led to an increase in the activity of Hypothalamus-Pituitary-thyroid axis and consequently increased the plasma thyroid hormones (mostly  $T_4$ ), (Currie, 1995), but because of the presence of greater active type I 5'-deiodinase in the treatment group than the control, more  $T_4$  converted to  $T_3$  and therefore it caused more  $T_3$  concentrations and less  $T_4$  concentrations in the treatment group than in control. These differences for  $T_3$  concentration continued until the fourth week of experiment.

Changes in rectal temperature in response to milk supplemented with Sel-Plex are shown in Figure 2A. Weekly comparison of the treatment with the control group showed that there was a significant increase in the rectal temperature in the third week of experiment, that our findings are different to some previous studies (Rock et al., 2001; Rowntree et al., 2004). Rock et al. (2001) found that rectal temperature of newborn lambs at birth and 12 hours of age were not affected by prenatal Se supplementation. Rowntree et al. (2004) reported that mean calf rectal temperature on 0, 3 and 7 days after birth was not influenced by maternal Se supplementation. In the current study, the existence of the effect of Sel-Plex treatment on the plasma ratio of  $T_3$  to  $T_4$ , FT<sub>3</sub>I and also on the rectal temperature of suckling calves indicated that 5'-deiodinase activity was induced in calves with access to selenium and caused more metabolism and more thermogenic activity, then higher rectal temperature in supplemented calves with Sel-Plex. Weekly comparison of rectal temperature of Sel-Plex group with the control showed that there was a significant increase in the amount of rectal temperature in the third week of experiment in the treated calves (Figure 2A). In the third week of experiment, in response to cold exposure, greater active thyroid hormones ( $T_3$ ) were produced by active type I 5'-deiodinase in Sel-Plex group

than in control group and it caused more metabolism, more heat production and higher rectal temperature in all tissues of the treated calves.

Sel-Plex supplementation reduced plasma Glucose concentrations ( $p < 0.01$ ) of calves with a marginal selenium status and comparing the Sel-Plex treatment group with the control one showed that there was a significant decrease in plasma glucose concentration in the last week of experiment (Figure 2B). This result was consistent with other studies (Mukherjee et al., 1998). Mukherjee et al. (1998) reported that Se supplementation reduces blood glucose levels and lipid peroxidation in streptozotocin-induced diabetic rats. Other several reports suggest an insulin-like effect for selenate in *in vitro* and *in vivo* experiments (McNeill et al., 1991; Becker, 1996; Stapleton, 2000). In our experiment, the effect of Sel-Plex on plasma glucose concentration may reflect the fact that Sel-Plex had insulin-like effect on suckling calves.

There was a significant decrease in cholesterol concentration during the third week of experiment in the treated calves with Sel-Plex (Figure 2C), which coincided with higher  $T_3$  concentration in the treated group. It has been indicated that thyroid hormones deprivation increased serum cholesterol concentration and treatment with L- $T_3$  (0.5 to 25 mg/mouse/day) caused a 57% decrease in serum cholesterol of mice. Serum levels of cholesterol seem to be mainly regulated by thyroid hormones action on liver (Roy et al., 1998). In the current study, selenium supplementation resulted in increase of plasma  $T_3$  concentrations and ratios of  $T_3$  to  $T_4$ . In the third week, therefore increase of  $T_3$  level probably led to reduction in plasma cholesterol concentrations.

Increasing Glutathione peroxidase activity of calves by supplementation of Sel-Plex is in agreement with other studies (Backall and Scholz, 1981; Gunter et al., 2003; Rowntree et al., 2004; Yue et al., 2009). Backall and Scholz (1981) reported that Holstein and Angus cows with adequate whole-blood selenium status (90 to 105 ng/ml) had whole-blood GSH-PX-1 activity between 27 to 35 enzyme units per gram of hemoglobin (EU/g of hemoglobin), whereas cows with marginal status had RBC GSH-PX-1 activity of 11.5 EU/g of hemoglobin. Therefore, in our study, calves in Sel-Plex group were in an adequate whole-blood glutathione peroxidase activity ( $40.7 \pm 3.9$  EU/g), and calves in control group were in marginal whole blood glutathione peroxidase activity ( $19.4 \pm 3.9$  EU/g). Gunter et al. (2003) reported British crossbred and Simmental cows in Arkansas consuming trace-mineralized salt with 26 mg/kg selenium as sodium selenite or yeast selenium for 4 months had RBC GSH-PX-1 activities of 101 or 106 EU/g of hemoglobin, respectively. These GSH-PX-1 activities in cows are considerably greater than those observed in our trial. Rowntree et al. (2004) reported that

the RBC GSH-PX-I activity of cows in both treatment groups (with or without selenium) did not differ during the first 3 months of study. However, after 4 months, selenium-drenched cows with 0.3 mg/kg selenium as sodium selenite had greater RBC GSH-PX-I activity and maintained this difference for the remainder of the trial (Rowntree et al., 2004). The delay in activity of RBC GSH-PX-I in response to selenium was likely due to the 90 to 120-d RBC life expectancy, resulting in only limited monthly incorporation of GSH-PX-I enzyme into RBC during erythropoiesis (Stowe and Herdt, 1992). In the present study, after 60 days Sel-Plex treatment, a significant increase in glutathione peroxidase activity was observed. Therefore, consuming Sel-Plex in suckling calves in shorter term leads to increase in glutathione peroxidase activity than mature cows. On the other hand, because the total period of our experiment was shorter than RBC life expectancy, the maximum activity of GSH-PX-I was not observed, and consequently we simply reported the amount of GSH-PX-I activity at the end of trial.

### IMPLICATIONS

Results indicated that Sel-Plex supplementation in milk of suckling calves with a marginal selenium status increased peripheral converting  $T_4$  to  $T_3$  and therefore change thermometabolism. These condition improved the capacity of suckling calves imposed to cold stress.

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