

# The Comparison Effect of Liquid Feeds and Sources of Dietary Selenium on Performance, Blood Metabolites and Anti-Oxidant Status of Holstein Neonatal Female Calves

Research Article

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## ABSTRACT

The objective of this study was to determine the effect of two sources of liquid feed and two sources of dietary selenium (Se) on the performance, blood metabolites and anti-oxidant status of Holstein neonatal female calves. Thirty Holstein neonatal female calves were allocated to their treatments, including the colostrum and milk as liquid feeds with adding 0.3 ppm of either sodium selenite (SS) or Se yeast (SY) as sources of Se and were compared in a 2 × 3 factorial design. Body weight, feed intake and skeletal growth parameters were measured by each calf at birth and at the end of the experiment (day 60). Blood samples were collected by jugular venipuncture into evacuated tubes containing EDTA and stored for further analysis. Data were subjected to analyze of variance with GLM using SAS (2004) and mean of parameters were comprised with Tukey test. The results of this experiment showed that although there was no significant effect of both liquid feeds and dietary Se on feed intake, the body weight gain and feed conversion ratio were significantly increased in calves fed by organic Se ( $P < 0.05$ ). Moreover, only body length and stomach size significantly increased in calves fed by organic Se ( $P < 0.05$ ). The results also showed no significant difference in plasma glucose and urea, while the mean of plasma Se, globulin and total protein concentration significantly increased in calves fed by organic Se ( $P < 0.05$ ). In addition, dietary Se not only had a significant effect on both glutathione peroxidase (GPX) and superoxide dismutase (SOD) activity but also had a significant effect on both growth hormone (GH) and insulin like growth factor (IGF-1), too ( $P < 0.05$ ). The results of the present study implied that adding 0.3 ppm of organic Se to milk or colostrum had a significant effect on performance, blood metabolites and anti-oxidant status of Holstein neonatal female calves.

**KEY WORDS** anti-oxidant status, blood metabolites, calf, colostrum, performance, selenium.

## INTRODUCTION

Se (Se) plays important role in several metabolic processes including antioxidant defense systems, thyroid hormone metabolism and immune function (Brown and Arthur, 2001). Because calves are always born with Se deficiency, feeding Se after birth is an important practice for promoting the development of their own immune system and thereby promoting their healthy growth. Se deficiency in calves

leads to several economic problems, the most important of which are lack of immunity, low of growth and fertility, abortions, low birth rates and low milk production. So that, an adequate dietary Se intake helps to prevent calves' disease caused by its deficiency, prevents the accumulation of lipid hydroperoxides in organs and tissues and thus protects them from damage by reactive oxygen species (ROS) (Bickhardt *et al.* 1999; Hodgson *et al.* 2006). Selenium is added to ruminant diets in the inorganic or organic form.

Sodium selenite is most frequently applied in the feed industry, which is commonly offered in mineral premixes. Selenized yeast (Se-Y) is the organic form of Se, this product is grown in high Se medium. Results obtained from ruminant species have demonstrated the greater bio efficacy of organic forms of Se compared with mineral forms (Juniper *et al.* 2006; Phipps *et al.* 2008; Gong *et al.* 2014). This improved bio efficacy of organic forms is directly related to the seleno methionine (SeMet) concentration of the Se supplement rather than its total Se concentration. In calves, Se concentration is associated with growth (Spears *et al.* 1986; Wichtel *et al.* 1996; Enjalbert *et al.* 2006) and morbidity (Waltner *et al.* 1986; Enjalbert *et al.* 2006; Waldner and Rossengren, 2009). Growth responses to Se supplementation are extremely variable. Some early research done by Burroughs *et al.* (1963), used Se supplementation at a rate of either 0.05 or 0.10 mg/kg to a ground corn based finishing diet, increased final body weight (BW) of beef steers by approximately 20 kg when fed for 141 days. Moreover, lambs fed a basal diet containing 0.19 mg/kg of Se with an additional 0.30 mg/kg of Se had increased average daily gain (ADG) and total gain during a 90 d study compared to lambs that only received the basal diet (Kumar *et al.* 2008). In addition, Growth has been defined as the production of new cells (Owens *et al.* 1993). Growth is not only limited to muscle, it encompasses adipose and bone tissue growth as well. Beef cattle are born with a set number of muscle fibers (Owens *et al.* 1993) meaning that post-natal muscle growth in cattle is through hypertrophy. Some research showed that growth hormone (GH) drastically reduced in animals treated with 15 ppm sodium selenite (5.0 ppm Se) in the drinking water (Thorlacius-Ussing *et al.* 1987). Growth hormone has been shown to increase the production and secretion of insulin-like growth factor-I (IGF-1), which ultimately results in increased muscle protein accretion (Florini, 1985). Insulin-like growth factor (IGF-1) is produced in skeletal muscle and is needed to stimulate protein synthesis and inhibit the rate of protein degradation in muscle cells (Dayton and White, 2008). It is also well established that IGF-1 facilitates the anabolic effects of GH on muscle and bone tissue (Dayton and White, 2008).

Moreover, it is well known that oxidative stress (an imbalance between free radical production and their detoxification) has a number of negative effect on antioxidant status, as well as animal growth, development and general health (Sordillo and Mavangira, 2014). Therefore, Se as a part of seleno proteins can be added to the animal diet to improve antioxidant defense. Some of the most important antioxidants are the glutathione peroxidase (GPx) which plays a pivotal role in the removal and detoxification of hydrogen and lipid peroxides and the superoxide dismutase (SOD),

which prohibits lipid peroxidation. Weiss and Hogan (2005) showed that, on average, Se yeast increased the whole blood GSH-Px activity more effectively than sodium selenite, but that in other studies, there was no difference (Knowles *et al.* 1999; Calamari *et al.* 2010). Because different sources of dietary Se may be utilized with different efficiencies and functions (Gunter *et al.* 2002), the objective of this study was to determine the effect of feeding Se sources and liquid feed sources on performance and antioxidant status of Holstein neonatal female calves.

## MATERIALS AND METHODS

### Experimental area and ethics

This study was conducted at a dairy research center (Faculty of Agriculture, Ferdowsi University of Mashhad) in Mashhad, Iran. All using procedures and animal handling were approved by the Agricultural Animal Care and Use Committee of the Ferdowsi University of Mashhad, that was done based on the guidelines of Iranian Council of Animal Care (1995).

### Experimental design

Before starting the experiment, the chemical composition of milk and colostrum was analyzed using a MilkoScan analyzer (Foss Electric A/S, Hillerod, Denmark) at the ruminant nutrition laboratory of Ferdowsi University of Mashhad (Mashhad, Iran). The chemical compositions of liquid feeds are shown in Table 1.

**Table 1** Chemical composition of the two liquid feeds

Composition (% of DM)	Colostrum	Milk
Total solid	19.5	12
Protein	8.1	3.3
Fat	6.2	3.5
Lactose	3.6	4.6
Minerals	1.1	0.8
Selenium (ppm)	0.05	0.02

A total of 30 Holstein neonatal female calves (mean weight: 38.45±0.1 kg) were selected for 60 day experimental period for this study. All female calves were separated from their mothers immediately after birth and then were placed in individual indoor pens and received fresh colostrum twice daily (1.5 to 2 kg per meal) for the first 2 days of life. On the third day, calves were switched to their treatments, including the colostrum and milk as liquid feeds with adding 0.3 (mg/kg) of either sodium selenite (SS) or Se yeast (SY) as sources of Se. Female calves received liquid feeds twice daily (2 kg per meal) and water and starters were available for *ad libitum* intake until the end of the experiment (day 60).

Colostrum was collected from the first 8 milking postpartum was diluted 1:1 (1 part of colostrum mixed with 1 part

of water) so that both colostrum and milk would have equal total solid (TS), fat, protein, and lactose contents. Sodium selenite as inorganic Se and Se yeast as organic Se supplement were provided by (Damyar jame Co, Ltd., Tehran, Iran). The quantity of the Se yeast and sodium selenite supplements was adjusted to equalize dietary Se supply between these 2 Se sources and equated to 0.3 mg/kg of Se per day. The concentration of Se in the colostrum and milk before adding Se was about 0.05 and 0.02 ppm and the doses of Se were dietary supplemented to the ratio. The compensatory amount for the nutritional requirement recommended by the [NRC \(2001\)](#) is 0.3 ppm of the Se for calves, because colostrum usually contains about 0.05 ppm or lower of Se, adding 0.3 ppm dietary Se is unlikely to be the limit to avoid poisoning as reported by [Jenkins and Hidiroglou \(1986\)](#). The composition and chemical characteristics of the starter mixture is clear in Table 2. The animal's requirements for CP and ME were calculated according to [NRC \(2001\)](#).

**Table 2** Composition and chemical characteristics of the starter

Ingredients	Starter
<b>Ingredient (% of DM)</b>	
Corn grain	50
Cotton meal	5
Soybean meal	21
Wheat bran	57
Barely grain	15
Di calcium phosphate	0.2
Calcium carbonate	0.8
Vitamin premix	1
<b>Chemical composition (% of DM)</b>	
Metabolizable energy (Mcal/kg of DM)	3.20
Crude protein	18.20
Calcium	0.8
Phosphorus	0.4
Selenium (ppm)	0.03

### Estimation of body size measurements

Neonatal calves were measured for growth to determine performance throughout the trial. After birth and at the end of the experiment, the skeletal growth parameters were measured: pin width, hip width, body length, heart girth, withers height, and hip height, by using a wooden height stick a horizontal crossbar and level, calibrated in 0.635-cm increments (actual calibrations were in English units). Body length (measured from point of shoulder to caudal projection of pin bone).

Body weight gain (BWG) was determined with a digital scale after birth and at the end of the experiment (day 60). Starter intake was also recorded daily by measuring feed provided and residuals. Fresh and clean water was available all time.

### Blood samples and estimation of blood components

Blood samples were collected both after birth and at the end of the experiment (day 60), by jugular venipuncture into evacuated tubes containing 0.1 mL of 15% EDTA (Vacutainer; Becton Dickinson, Rutherford, NJ), placed on ice, and transported to the laboratory. Plasma was harvested by centrifugation at  $3000 \times g$  for 15 min at 4 °C and stored at -20 °C until analyzed for glucose, urea, Se, globulin and total protein concentration, Glutathione peroxidase and superoxide dismutase activities, growth hormone (GH) and insulin-like growth factor-I (IGF-1). Plasma glucose concentrations were measured by the glucose oxidase method using a commercial kit (No. 115; Sigma Diagnostics, St. Louis, MO). Plasma urea nitrogen (PUN) concentration was determined using urease and the indophenol reaction ([Chaney and Marbach, 1962](#)).

Urea standards were prepared by dissolving 0.2142 g of urea in 100 mL of deionized water to form a stock solution and then further diluting the stock with deionized water to prepare a set of standards. The activity of Glutathione peroxidase (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 \(1985\)](#).

The serum super-oxide dismutase (SOD) activity was measured using commercial colorimetric assay kits (Nanjing Jian- cheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The absorbance was measured using a spectrophotometer at various wave- lengths (UN2CO-WFT2100, Shanghai, China). Plasma insulin-like growth factor-I (IGF-1) concentration was measured by a commercial 125I-RIA Kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). Plasma concentrations of growth hormone (GH) were determined by the double antibody RIA technique, as described by [Barnes \*et al.\* \(1985\)](#). Plasma were analyzed for Se concentrations using inductively coupled plasma mass spectroscopy (PerkinElmer, Waltham, MA) as described by [Pogge \*et al.\* \(2012\)](#) and concentration of both serum total protein (TP) and Serum globulins were determined by spectrophotometer (Unico, USA) using commercial test kits.

### Statistical analysis

Two liquid feeds and two sources of Se were compared in a  $2 \times 3$  factorial design during the 60-d period of the experiment. Data were subjected to ANOVA with a general linear model (GLM) using the MIXED procedure of [SAS \(1996\)](#) and the mean of parameters were analyzed by the Tukey test. Significance was declared at  $P < 0.05$ , unless otherwise

indicated. Least squares means were separated using the PDIF option of SAS. The results were expressed in the tables as the least square mean and if there were significant interactions, will be present in the tables and if is not will be omitted.

## RESULTS AND DISCUSSION

The present study was focused on the effect of both liquid feeds and dietary Se on neonatal female calves' performance traits, such as feed intake, body weight and other body measurements including pin width, hip width, body length, heart girth, withers height, and hip height. The results showed that there were no significant effects of both liquid feeds and sources of Se on calves' feed intake throughout of the study (Table 3). This lack of effect is not surprising as several studies on calves have reported no significant response to supplemental Se to feed intake (Gunter *et al.* 2003; Stockdale and Gill, 2011; Cortinhas *et al.* 2010). Similarly, Vignola *et al.* (2009) reported that Se supplementation to lambs in addition to a basal diet containing 0.13 mg Se/kg DM in the forms of Sodium selenite (0.30 mg Se/kg), or Se-yeast (0.30 mg Se/kg, or 0.45 mg Se/kg) had no effect on feed intake. According to our results, the average of total and daily feed intake per calf for No-Se, sodium selenite, and Se yeast treatments were (40.2, 38.8 and 39.7 kg) and (0.674, 0.651 and 0.661 kg) during the whole experimental period, respectively. In No-Se treatments, the slight increase in feed intake maybe due to more nutrient requirements that lead to increased feed consumption and thereby the calves automatically get a higher feed intake without any Se supplementation which is confirmed by (Stowe and Heardth, 1992).

Total BWG and ADG were also significantly increased and feed conversion ratio (FCR) improved in Se-supplemented calves compared to the control ( $P<0.05$ ). Both of total BWG and ADG for the SY, SS and No-Se treatments were (24.60 and 21.27 vs. 18.78 kg) and (0.409 and 0.354 vs. 0.312 kg), respectively. Some early research done by Burroughs *et al.* (1963) reported that Se supplementation at a rate of either 0.05 or 0.10 ppm to a ground corn based finishing diet, increased final BW of finishing beef steers by approximately 20 kg when fed for 141 days. Lambs fed a basal diet containing 0.19 mg/kg of Se with an additional 0.30 mg/kg of Se had increased ADG and total gain during a 90 d study compared to lambs that only received the basal diet (Kumar *et al.* 2008). In addition, also observed that the ADG of heifers supplemented with 0.2 ppm Se in their concentrate mixture, was significantly ( $P<0.05$ ) higher compared to the others. It indicated that Se supplementation affects growth mainly during the early stage of growth, and this may be attributed to its contribu-

tion in selenoprotein synthesis and their role in thyroid hormone synthesis, which may be responsible for high metabolism and better weight gain (Underwood and Suttle, 2004). On the other hand, the lower BWG on control treatments is considered to be linked to the interaction of Se with thyroid hormones. Other mammals have an enzyme pathway allowing the production of T3 thyroid hormone even in low Se situations, however this does not exist to any appreciable extent in cattle (Arthur *et al.* 1993), making them more susceptible to the growth limitations of a low Se diet. Also observed an increase in growth rate in lambs supplemented with 5 mg Se/month and 1 mg Se/kg live weight. Similarly, Oblitas *et al.* (2000) found significant ( $P<0.05$ ) increase in weight gain in Holstein heifers supplemented with a single dose of 5 mg Se/100 kg body weight. Findings of present study are also supported by Wichtel *et al.* (1996) and Kumar *et al.* (2008), who observed higher weight gain and ADG on supplementation of either intraruminal Se pellets (designed to release about 3 mg Se /day) to calves or in the diet of lambs 0.15 and 0.3 ppm. In contrast, Nicholson *et al.* (1993) reported no growth response to Se supplementation from a variety of sources, at rates ranging from 0.03- 1.2 mg Se/kg DM with a basal diet containing 0.02 mg Se/kg DM, when given to growing beef steers and Holstein heifers for 112 day. Arthur *et al.* (1990) also showed no significant change in BW gain in response to Se supplementation.

Se-supplemented calves showed a significantly better feed conversion ratio (FCR) than control (No-Se) animals (1.636 and 1.911 vs. 2.171), respectively ( $P<0.05$ ). FCR is a direct method to evaluate feed conversion efficiency (Song *et al.* 2014).

The smaller FCR values on Se treatments indicate higher efficiency of feed utilization, which is good for animal growth and fattening. We observed lower FCR values for Se yeast added groups, which suggest that the addition of Se yeast could improve the efficiency of feed conversion. Similarly, Wichtel *et al.* (1996) reported that Supplement Se-yeast and Na-selenite to rations was improved significantly feed conversion ratio of growing buffalos. This may be due to positive effect of Se source on nutrient digestibility or due to the tendency increase on daily BWG of supplements groups. In contrast, Hidiroglou and Jenkins (1975) added 0.2, 1.0, 3.0, 5.0 and 10 ppm of sodium selenite to calf milk replacer and found no difference in feed efficiency up to a concentration of 5 ppm, however, a decrease was observed at 10 ppm.

Juniper *et al.* (2009) observed no difference in the performance of calves when they added 10 times the maximum Se-yeast amount allowed by the European Union for calf feed (0.15 to 5.6 mg of Se/kg DM) due to concerns about possible toxicity.

**Table 3** Effect of two liquid feeds and two sources of selenium on feed intake and performance of Holstein neonatal female calves

Item (cm)		Treatment						Liquid effect		Selenium effect			SEM	P-value	
		C			M			C	M	No-Se	SS	SY		Liquid	Selenium
		No-Se	SS	SY	No-Se	SS	SY								
BW (kg)	Day 1	39.40	39.20	40.40	37.40	38.74	35.58	39.66	37.24	38.40	38.97	37.99	1.32	0.20	0.91
	Day 60	57.00	60.06	65.24	57.36	60.42	59.94	60.77	59.24	57.18	60.24	62.59	1.99	0.51	0.17
Total BW gain (kg)		17.60	20.86	24.84	19.96	21.68	24.36	21.10	22.00	18.78	21.27	24.60	1.22	0.52	0.01
ADG (kg/d)		0.293	0.347	0.414	0.332	0.361	0.406	0.351	0.366	0.312	0.354	0.409	0.01	0.52	0.01
Feed intake (kg)	Total	40.00	38.20	39.40	40.40	39.40	40.00	39.20	39.93	40.20	38.80	39.70	0.48	0.52	0.13
	Daily	0.666	0.636	0.656	0.683	0.666	0.666	0.653	0.671	0.674	0.651	0.661	0.06	0.06	0.10
FCR		2.291	1.889	1.623	2.052	1.933	1.650	1.934	1.878	2.171	1.911	1.636	0.10	0.65	0.01

C: colostrums; M: milk; No-Se: control; SS: sodium selenite and SY: selenium yeast.  
 BW: body weight; ADG: average daily gain and FCR: feed conversion ratio.  
 SEM: standard error of the means.

There were no differences on skeletal growth parameters among treatment groups, however, the total increase of both body length and heart girth were significantly increased ( $P < 0.05$ ) by (12.7, 11.8 and 8.8 cm) and (22.5, 22.66 and 17.8 cm) on calves fed by SY, SS and No-Se at the end of the experiment, respectively (Table 4). Moallem *et al.* (2004) reported a significant increase in the average daily gain for calves treated by Se injection (0.05 mg/kg on day 2, 70, 114 and 149 days) as compared to controls. He mentioned that because growth rates were limited by feed intake, adding dietary Se to liquid feeds, may supply more nutrients to calves and leads to heavier weight gain and larger body dimensions. In the present study, the higher body weight and consequently larger body dimensions may be due to more efficient use of nutrients on calves fed by dietary Se, as these growth parameters are indicative of an improvement in the development of the animals. The same result was observed in Shamay *et al.* (2005), who indicated better skeletal growth during the rearing period may be associated with the genetic potential and nutrition of the animals.

Searle *et al.* (1989) also reported that sheep had higher feed intake, had larger body dimensions (e.g. hip height, leg length, shoulder height) because of consumption more nutrients. Thus, undernourished animals tend to have shorter body size, slower growth rate and lower body weight, too. On the other hand, both of lower body weight and skeletal growth parameters in No-Se groups, suggest an impaired metabolic utilization of nutrients in Se-deficient animals as observed by (Adkins and Ewan, 1984). So that, it can be concluded that Se does not play a direct role in promoting skeletal growth rate in calves, but it helps to remove all constraints that may delay or inhibit their growth rate.

Se supplementation either with organic or inorganic form did not affect significantly on plasma glucose concentration as shown in Table (5).

However, plasma glucose concentration tended to be slightly lower in No-Se calves than those fed by organic and inorganic Se. These results were in agreement with that of Calamari *et al.* (2011) and Tahmasbi *et al.* (2012) who found that dairy cows treated with sodium selenite or organic Se as well as injection of Se-vitamin E had no significant effect on their plasma glucose. Similarly, Kamada (2017) observed no significant rise in plasma glucose levels in Holstein cows which were fed yeast based Se at the rate of 0.3 mg/kg dry matter intake. However, there is a much discrepancy in the glucose response of dairy animals supplemented with Se.

For example, contrary to our findings, Singh *et al.* (2002) reported a low concentration of glucose in the blood of buffalo calves offered wheat straw having high Se (8.54 mg/kg DMI).

Nayyar *et al.* (2003) also found that blood glucose levels in buffalo heifers subjected to vitamin E-Se was significantly higher than control group. These results may be due to the nature of Se, dose, duration and other experimental protocols.

Results illustrated in Table 5 revealed that plasma urea concentration was not affected significantly by both liquid feeds and Se sources, however calves fed by Se sources had a slightly lower plasma urea concentration than the others. The insignificant effect of both organic and inorganic Se treatments was followed by Tahmasbi *et al.* (2012) who found that treated dairy cows by injection of Se-vitamin E had no significant effect on their plasma urea. Urea concentration in plasma is the result of the balance between urea input (i.e., produced by the liver and rumen utilization and absorption of urea) and output (i.e., elimination by the kidneys and passive loss via feces) (Weiner *et al.* 2015). Hence, increased plasma urea can be caused by increased urea production, decreased urea elimination, or a combination of the two (Higgins, 2016).

**Table 4** Effect of two liquid feeds and two sources of selenium on skeletal growth parameters of Holstein neonatal female calves

Item (cm)	Treatment												SEM	P-value	
	C			M			Liquid effect		Selenium effect			Liquid		Selenium	
	No-Se	SS	SY	No-Se	SS	SY	C	M	No-Se	SS	SY				
Pin width	Day 1	9.00	9.60	9.80	8.60	9.20	9.40	9.46	9.06	8.80	9.40	9.60	0.28	0.23	0.13
	Day 60	13.60	13.20	13.80	12.20	12.60	13.40	13.53	12.73	12.90	12.90	13.60	0.39	0.09	0.37
	Total increase*	4.60	3.60	4.00	3.60	3.40	4.00	4.06	3.66	4.10	3.50	4.00	0.26	0.20	0.24
Hip width	Day 1	14.80	15.40	15.40	14.80	15.00	15.60	15.20	15.13	14.80	15.20	15.50	0.48	0.90	0.60
	Day 60	20.40	19.80	20.00	19.40	20.00	20.60	20.06	20.00	19.90	19.90	20.30	0.55	0.91	0.84
	Total increase	5.60	4.40	4.60	4.60	5.00	5.00	4.86	4.86	5.10	4.70	4.80	0.32	1.00	0.66
Body length	Day 1	60.60	61.20	60.40	58.80	58.40	57.60	60.73	58.28	59.70	59.80	59.00	1.10	0.06	0.85
	Day 60	68.40	72.00	73.40	68.60	71.20	70.00	71.26	69.93	68.50	71.60	71.70	0.65	0.09	0.01
	Total increase	7.80	10.80	13.00	9.80	12.80	12.40	10.53	11.66	8.80	11.80	12.70	0.83	0.25	0.01
Heart girth	Day 1	80.00	81.60	82.00	80.00	81.20	77.80	81.20	79.67	80.00	81.40	79.90	1.74	0.45	0.79
	Day 60	98.00	103.40	104.40	97.60	104.60	100.40	101.93	100.87	97.80	104.00	102.40	1.65	0.58	0.03
	Total increase	18.00	21.80	22.40	17.60	23.40	22.66	20.73	21.20	17.80	22.66	22.50	0.61	0.51	0.03
Withers height	Day 1	64.70	67.40	69.80	69.00	67.00	70.80	67.33	68.93	66.90	67.20	70.30	1.29	0.29	0.14
	Day 60	78.80	80.80	82.40	81.40	82.60	85.00	80.67	83.00	80.10	81.70	83.70	1.00	0.06	0.06
	Total increase	14.00	13.40	14.60	12.40	15.60	14.20	14.00	14.07	13.20	14.50	14.40	0.88	0.94	0.52
Hip height	Day 1	68.20	71.00	73.60	72.40	70.80	74.20	70.93	72.47	70.30	70.90	73.90	1.47	0.37	0.20
	Day 60	83.20	86.60	86.60	87.60	86.60	89.00	85.47	87.73	85.40	86.60	87.80	1.24	0.12	0.41
	Total increase	17.00	15.60	13.00	15.20	15.80	14.80	15.20	15.27	16.10	15.70	13.90	0.81	0.84	0.14

\* The total increase of body dimensions between day 1 and day 60.

C: colostrums; M: milk; No-Se: control; SS: sodium selenite and SY: selenium yeast.  
SEM: standard error of the means.

**Table 5** Effect of two liquid feeds and two sources of selenium on blood metabolites, growth hormones and anti-oxidant status of Holstein neonatal female calves

Item	Treatment												SEM	P-value	
	C			M			Liquid effect		Selenium effect			Liquid		Selenium	
	No-Se	SS	SY	No-Se	SS	SY	C	M	No-Se	SS	SY				
Glucose (mg/dL)	Day 1	115.60	93.20	90.20	96.00	103.60	96.40	99.67	98.67	105.80	98.40	93.30	3.18	0.24	0.31
	Day 60	163.60	156.60	138.60	144.20	130.00	163.80	152.93	146.00	153.90	143.30	151.20	4.08	0.24	0.31
Urea (mg/dL)	Day 1	18.60	18.40	17.60	22.60	18.00	19.20	18.20	19.93	20.60	18.20	18.40	0.47	0.17	0.13
	Day 60	22.60	23.40	21.60	26.40	22.60	24.80	22.53	24.60	24.50	23.00	23.20	0.62	0.29	0.34
Selenium (ng/mL)	Day 1	75.80	73.00	74.00	76.20	75.20	73.80	74.27	75.07	76.00	74.10	73.90	0.52	0.29	0.07
	Day 60	109.20	108.60	112.40	109.00	117.20	117.20	110.07	114.47	109.10	112.90	114.80	1.18	0.01	0.03
Globulin (g/dL)	Day 1	2.48	2.40	2.44	2.70	3.24	3.26	2.44	3.06	2.59	2.82	2.85	0.21	0.10	0.75
	Day 60	2.76	3.02	3.74	3.30	3.70	4.18	3.17	3.72	3.03	3.36	3.96	0.18	0.04	0.02
Total protein (g/dL)	Day 1	4.14	4.22	4.00	4.28	4.06	4.16	4.12	4.16	4.21	4.14	4.08	0.06	0.53	0.36
	Day 60	6.10	6.52	6.70	6.22	6.36	6.74	6.44	6.44	6.16	6.44	6.72	0.08	0.90	0.05
GPX (U/mL)	Day 1	163.20	146.40	175.00	131.40	172.00	172.60	161.53	160.00	147.30	159.20	175.80	8.64	0.87	0.09
	Day 60	182.80	190.20	204.80	179.40	199.40	225.00	192.60	201.27	181.10	194.80	214.90	8.07	0.36	0.02
SOD (U/mL)	Day 1	47.60	48.00	49.20	48.40	52.40	47.40	48.27	49.40	48.00	50.20	48.30	1.10	0.47	0.40
	Day 60	77.60	84.20	88.80	75.40	85.00	87.80	83.53	82.73	76.50	84.60	88.30	2.05	0.73	0.01
GH (ng/mL)	Day 1	38.20	38.24	39.00	37.58	38.08	37.96	38.48	37.87	37.89	38.16	38.48	0.53	0.43	0.81
	Day 60	51.68	56.80	64.28	51.66	56.96	57.20	57.59	55.27	51.67	56.88	60.74	1.06	0.07	0.01
IGF-1 (ng/mL)	Day 1	122.26	123.56	126.22	123.62	121.72	121.64	124.01	122.33	122.94	122.64	123.93	1.34	0.38	0.84
	Day 60	202.04	215.62	239.42	195.24	223.82	237.50	219.03	218.85	198.64	219.72	238.46	2.31	0.90	0.01

C: colostrums; M: milk; No-Se: control; SS: sodium selenite and SY: selenium yeast.

GPX: glutathione peroxidase activity; SOD: super oxide dismutase activity; GH: growth hormone concentration and IGF-1: insulin like growth factor concentration.  
SEM: standard error of the means.

Moreover, Farghaly *et al.* (2017) reported that blood urea concentration were significantly ( $P < 0.05$ ) decreased with supplement Se-yeast and Na-selenite as compared with control on growing buffalo calves. In the present study, the lower plasma urea concentration on calves fed by Se sources compared to other calves, may be due to their lower feed and protein intake. As Canfield *et al.* (1990) concluded that higher feed intake of the cows will result in higher blood urea nitrogen concentration. In contrast, an increase in blood urea concentration in Se supplemented animals was observed in rats supplemented with inorganic Se (Abdo, 1994) and in one year old Angus steers fed organic Se (Jia *et al.* 2019) but not in multiparous dairy cows

(Kamada, 2017; Khalili *et al.* 2019).

The results of protein fractions and Se concentration of calves are shown in Table 5. The results of the present study showed that calves fed by Se sources had a significantly higher Se concentration in plasma compared with others ( $P < 0.05$ ). In addition, plasma Se concentration in the calves supplemented with Se-yeast were higher than those received Na-selenite (114.8 vs. 112.9 ng/mL), which supports the general view that organic Se is more effective than inorganic Se at increasing blood Se concentrations (Givens *et al.* 2004; Juniper *et al.* 2009). This difference has been described by the metabolism of inorganic and organic Se with regard to absorption and post-absorption metabolism.

**Table 6** Comparison of BW gain, FCR, plasma Selenium, GH and IGF-1 concentration of Holstein neonatal female calves

Item		Treatment						Selenium effect			SEM	P-value Selenium
		C			M			No-Se	SS	SY		
		No-Se	SS	SY	No-Se	SS	SY					
Selenium (ng/mL)	Day 1	75.80	73.00	74.00	76.20	75.20	73.80	76.00	74.10	73.90	0.52	0.07
	Day 60	109.20	108.60	112.40	109.00	117.20	117.20	109.10	112.90	114.80	1.18	0.03
BW (kg)	Day 1	39.40	39.20	40.40	37.40	38.74	35.58	38.40	38.97	37.99	1.32	0.91
	Day 60	57.00	60.06	65.24	57.36	60.42	59.94	57.18	60.24	62.59	1.99	0.17
FCR	Day 1	2.27	1.833	1.561	2.02	1.87	1.55	2.14	1.85	1.56	0.1	0.01
	Day 60	38.20	38.24	39.00	37.58	38.08	37.96	37.89	38.16	38.48	0.53	0.81
GH (ng/mL)	Day 1	51.68	56.80	64.28	51.66	56.96	57.20	51.67	56.88	60.74	1.06	0.01
	Day 60	122.26	123.56	126.22	123.62	121.72	121.64	122.94	122.64	123.93	1.34	0.84
IGF-1 (ng/mL)	Day 1	202.04	215.62	239.42	195.24	223.82	237.50	198.64	219.72	238.46	2.31	0.01
	Day 60											

C: colostrums; M: milk; No-Se: control; SS: sodium selenite and SY: selenium yeast.

BW: body weight; FCR: feed conversion ratio; GH: growth hormone concentration and IGF-1: insulin like growth factor concentration.

SEM: standard error of the means.

It is claimed that the absorbed inorganic Se is quickly transformed into metabolically available selenide and may be converted by selenophosphates into functional selenoproteins or excreted in the feces, while organic Se is absorbed through the absorption system of amino acids and its metabolism is closely linked to protein turnover (Schrauzer, 2003). Some previous studies suggested that Se status is adequate if its serum concentration in dairy cows and calves is in the range of 70 to 79 µg/L and 80 to 300 µg/L, respectively (Gong *et al.* 2014; Rossi *et al.* 2017). Se status is also an important indicator of animal health, and this is commonly assessed by measuring Se concentration in whole blood or serum and plasma (Pavlata *et al.* 2011). According to Kamada (2017), calves are deficient in Se at birth and mineral supplementation at this stage of life promotes an immediate supply of Se, which contributes to the development of the animal's immune system.

The value of globulin was significantly ( $P < 0.05$ ) higher in both liquid feeds and Se supplemented groups than control one. The higher value of globulin found with supplemented Se groups may be due to the effect of Se supplementation, which increase total serum globulins (Hoffmann and Berry, 2008). The effects of Se supplementation on immune responses of neonatal calves have also been scarcely investigated. Several studies have evaluated the effects of this mineral on immunoglobulin concentration in newborn calves (Abdelrahman and Kincaid, 1995; Rowntree *et al.* 2004; Weiss and Hogan, 2005; Kamada *et al.* 2007). The amount of Se in rations for young calves that are recommended by the NRC (2001) is 0.30 mg/kg DM. For some reason, the administration of Se together with milk and colostrum contributed to this immunological process either by facilitating the absorption and metabolism of the mineral or through a combination with some nutrient present in milk and colostrum but not available in the calves ration and this configuration can improve the calves' immune systems because immunoglobulins are involved in the defense of the organism against infections.

Maggini *et al.* (2007) reported that Se affects the immune system, influence humoral immunity mechanisms and increase the levels of globulin on pre weaning calves. Se supplementation also improves the immune function of the cows (Sordillo and Mavangira, 2014) and the amount of immunoglobulins in the colostrum, potentially benefitting the calves (Hall *et al.* 2013). Moreover, some studies also have evaluated the relationship between Se and increased blood globulin concentration in newborn calves of dams supplemented with the mineral before calving (Rowntree *et al.* 2004; Weiss and Hogan, 2005; Guyot *et al.* 2007). Kamada *et al.* (2007) also observed increases of Se and immunoglobulin G in the blood of calves fed colostrum supplemented with Se.

The results of this study showed a significant ( $P < 0.05$ ) effect of dietary Se sources on concentration of total plasma proteins (Table 5). At the early age, the plasma concentration of protein depends on many factors, including the amount of milk and colostrum fed and protein consumed. As calves get older, the plasma protein level is less dependent on colostrum and milk and more on plasma Se level (Quigley *et al.* 1998). Numerous studies found correlation between total protein concentration and immunoglobulin in calves (Moore *et al.* 2005). In the present study, the higher concentration of total protein, globulin and Se plasma on calves fed by Se implied an interesting specific relationships between them as reported by (Droke and Loerch, 1989). He mentioned that total plasma protein levels were positively related to Se levels. Albumin and globulin typically constitutes 50% and 35% of the total plasma proteins that found to be positively related to Se levels. It seems likely, therefore, that plasma protein increases were accelerated largely due to the influence of albumin and globulin, which were specifically affected by Se. Moreover, Mudgal *et al.* (2007) observed significantly higher level of globulin and total protein in male buffalo calves on supplementation of 0.3 mg Se per kg DM as the same result obtained in this study.

Thus, it appears that adding both inorganic and organic Se in liquid feeds can improve the levels of blood proteins in neonatal calves. In contrast, Kumar *et al.* (2008) reported that supplementation of inorganic or organic Se sources had no effect on serum protein, albumin and globulin of lambs. Arthur *et al.* (1993) and Singh *et al.* (2002) also did not find any significant difference in the total protein and globulin concentration in steers and buffalo calves supplemented with 0.1 and 8.54 mg Se per kg DM, respectively.

Significant increases in plasma GSH-Px activity was found among all the calves fed by Se sources ( $P < 0.05$ ) and the Se-yeast group showed the highest activity, the Na-selenite group an intermediate activity and the No-Se group had the lowest activity (214.9 and 194.8 vs. 181.1 U/mL) respectively. Similar to the current study, other researchers have reported increased erythrocyte GPX activity, plasma GSH-Px activity, or both in Se diets fed to swine (Mahan *et al.* 1999), steers (Hintze *et al.* 2001), and cows (Ortman and Pehrson, 1999; Gunter *et al.* 2003). However, plasma GSH-Px activity appears to be similar when supplemental Se is provided via inorganic or organic Se (Mahan *et al.* 1999; Ortman and Pehrson, 1999). Glutathione peroxidase is an enzyme that contains Se as selenocysteine in its catalytic site and which catalyzes hydro peroxide detoxification, metabolizing hydrogen peroxide and lipid peroxides to water and harmless oxy compounds (Xu *et al.* 2007). Se status is usually evaluated directly by determining the concentration of Se, or indirectly by measuring the activity of GSH-Px in blood and tissues (Gong *et al.* 2014). Whereas whole blood or erythrocyte GSH-Px reflects long-term Se status, serum or plasma GSH-Px reflects short-term Se status (Rowntree *et al.* 2004), and blood Se concentration is a good indicator of blood GSH-Px activity (Knowles *et al.* 1999). Results of the present study showed that, adding 0.3 mg/kg of DM Se, significantly increased the activity of plasma GSH-Px and also showed that Se-yeast is more effective at supporting antioxidant defense systems than Na-selenite (Table 5). The higher value of GSH-Px activity among calves fed by Se-yeast compared those fed by Na-selenite, can be caused by differences in the metabolism of the forms of Se or by its function as antioxidant enzymes. As indicated by Weiss and Hogan (2005), dietary selenomethionine more efficiently absorbed at the gut through a methionine carrier while, most of the Na-selenite is converted into low-absorbable compounds. After absorption, seleno-methionine can be incorporated into proteins or catabolized and the Se utilized to synthesize seleno enzymes like GPx, but selenite is reduced to selenide and converted into selenocysteine that is methylated and excreted. Similar results were also reported by Gong *et al.* (2014), who compared the effects of the same level of supplementation with Na-selenite and Se-yeast (0.3 mg/kg of

DM) on the antioxidant status of dairy cows fed basal diets containing Se at 0.02 mg/kg of DM, and found that organic Se increased serum GSH-Px activity more efficiently than inorganic Se. He mentioned that the plasma GSH-Px activity responded to changes in the dietary Se intake more rapidly than either the whole blood or erythrocyte GSH-Px activity (Thompson *et al.* 1998). Moreover, some studies reviewed by Weiss and Hogan (2005) and subsequent studies reported by Juniper *et al.* (2006) and Phipps *et al.* (2008) noted higher GSH-Px activity in cows receiving Se-yeast compared with those receiving Na-selenite. However in contrast, Calamari *et al.* (2010) reported that the whole blood GSH-Px activity was not affected by the dietary Se sources.

During the entire experimental period, the Se supplementation significantly increased the serum SOD activity of calves compared to the control group ( $P < 0.05$ ). The same results were also observed in dairy cows (Xu *et al.* 2007; Luo *et al.* 2010) and in chicken (Ahmad *et al.* 2012). Gong *et al.* (2014) reported that Se supplementation increased the serum SOD activity of dairy cows. He also mentioned that there were positive correlations between the SOD activity, serum Se and Cu concentration in goats (Kachuee *et al.* 2013) and calves (Al-Qudah *et al.* 2010), so this may explain why the serum SOD activity had an increasing trend in the Se supplementation groups in the present study. Moreover, SOD is considered to be an important marker that reflects the antioxidant status of animals (Gong *et al.* 2014). In the present study, supplementation of Se-yeast on calves' diet significantly increased the activity of SOD compared with Na-selenite and No-Se groups at 0.3 mg of Se/kg of DM. Therefore, supplementation with organic Se improved antioxidant status in calves' more than inorganic Se. These results corroborate the findings of Gong *et al.* (2014), who found that Se-yeast was more effective at improving antioxidant status than Na-selenite. In addition, the role of intracellular SOD is to scavenge the superoxide ( $\bullet\text{O}_2^-$ ) that is produced by a number of reaction mechanisms, including several enzyme systems, as a part of normal cellular functions. SOD also catalyzes the dismutation of ( $\bullet\text{O}_2^-$ ) into oxygen and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and it is an important antioxidant defense mechanism in aerobic organisms, although too much SOD may sometimes be deleterious (Halliwell and Chirico, 1993). In fact, the dismutation of ( $\bullet\text{O}_2^-$ ) results in a rise in  $\text{H}_2\text{O}_2$ . Since SOD activity increases  $\text{H}_2\text{O}_2$  production, protection from reactive oxygen would only be conferred by a coordinate increase of catalase and GSH-Px activities (Clemens and Waller, 1987; Frei, 1994; Kehrer and Smith, 1994). In support of this conjecture, GSH-Px activity was also found to be increased in calves fed by Se sources as obtained in this study. So that, we speculate that the coordinate increase of plasma SOD



and GSH-Px activity may be an indirect compensatory response of cells to increased oxidant challenge during early age of calves. On the other hand, the increased antioxidant enzyme activities may be due to the fact that pre weaning period represents an oxidative challenge for neonatal calves and adding Se supplementation causes improvement in the antioxidant status of them. However, the exact reason is still unclear, and further studies are necessary to explore the probable mechanism.

Overall mean of growth hormone concentration (GH) significantly increased on calves fed by Se sources ( $P < 0.05$ ) (Table 5). No significant differences were observed between the liquid feed treatments during the whole experimental period and the highest level of GH concentration was achieved by Se-yeast treatment (60.74 ng/mL). Similar results are reported in both mineral Se supplementation (Na-selenite) and organic (Se-yeast) in the cow and calf diets (Swecker *et al.* 1989; Awadeh *et al.* 1998; Gunter *et al.* 2003). The increasing GH concentration by Se supplementation may be due to the fact that thyroid hormones increase the levels of mRNA in the pituitary cells to secrete the growth hormone (Moav and McKeown, 1992; Farchi-Pisanty *et al.* 1995), or the role of Se by raising the concentration of growth hormone through its associated with an increase in the activity of deiodination to convert thyroid hormone from T4 to T3 which is necessary to form the hormone naturally in the pituitary gland or to the activity of the thyroid gland which is Se considered an essential part of the deiodinase enzyme that considered component of their necessary hormones for the secretion of the natural growth hormone of the pituitary gland to many mammal species (Valcavi *et al.* 1992; Muller *et al.* 1999). On the other hand, Se is involved in the metabolism of thyroid hormones. So that, a Se-deficient diet causes a reduction of triiodothyronine (T3) and an increase of the tetraiodothyronine (T4) and a decrease in the ratio T3/T4 levels in blood (Hidioglou *et al.* 1975; Beckett *et al.* 1989). These effects can influence growth hormone since T3 is an active form of T4 which is known to be involved in the growth hormone mechanisms. The activation of the T4 is done using the enzyme 5-iodothyronine deiodinase. This seleno-dependent seleno protein is one of the last proteins to be affected in the event of Se deficiency. This delay could explain the fact that several studies that have explored various ways of Se supplementation do not show any significant effect of Se supplementation on growth hormone and weight gain of calves, cows or bulls. In addition, the lowest GH concentration observed in No-Se group (51.67 ng/mL) may be due to the fact that, other mammals have an enzyme pathway allowing the production of T3 thyroid hormone even in low Se situations, however this does not exist to any appreciable extent in cattle (Arthur *et al.* 1993), making them more susceptible

to the growth limitations of a low Se diet. However, In contrast of our result, Arthur *et al.* (1990) reported that Se supplementation had not any significant effect on calves' growth hormone. He suggested that the effect of Se deficiency in calves may not be mediated by alterations in peripheral growth hormone concentration. He also mentioned that peripheral GH concentration did not change in Se deficiency, suggesting that Se could alter somatotrophic function via 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, the significantly positive effect of Se on the GH concentration of calves may be mediated by increased activity of type II 5'-deiodinase and greater synthesis of IGF-I.

Plasma insulin like growth factor 1 (IGF-1) in calves fed Se sources was significantly higher ( $P < 0.05$ ) from those calves fed No-Se (Table 5). Though there was no significant effect on calves fed by liquid feeds, only a small rise of plasma IGF-I concentrations was seen on colostrum treatments compared milk treatments (219.03 vs. 218.80 ng/mL). This effect may be due to less nutrients and energy of milk than colostrum during the experimental period and it seems that nutrient intake play a major role in regulation of the IGF-1 status as the same result observed in studies of Ronge and Blum (1988), Baumrucker *et al.* (1992), and Lee *et al.* (1995). Moreover, our results also showed that calves fed by Se-yeast had the highest IGF-1 concentration, the Na-selenite had the intermediate concentration and the control group had the lowest IGF-1 concentration (238.46 and 219.72 vs. 198.64 ng/mL). The same result observed by Xiaoying *et al.* (2011) who found that Nano-size Se supplemented on goats (basal diet with 0.5 mg/kg) significantly increased the IGF-1 compared control group. Similarly, reported that Se intake is important for the production and bioactivity of IGF-1. Insulin-like growth factor is a peptide hormone produced in the liver, which is formed as a consequence of growth hormone (GH) release from the pituitary gland, which stimulates subsequently IGF-1 production in the liver. IGF-1 is therefore a mediator for some of the GH functions, thus involved in growth and anabolism of calves. On the other hand, dietary Se status is a major regulator of the production and action of GH and IGF-I, and some of the observed effects of Se supplemented on growth and on the GH-IGF-I system may be related to better protein absorption in Se supplemented calves (Thissen *et al.* 1994; Ammann *et al.* 2000).

Our results showed that plasma Se, GH and IGF-I concentration are positively correlated with BWG and Se supplemented was associated with an increase on calves' body weight (Table 6). Increases in BWG in up to 25% following Se supplementation and a trend to increased concentrations

of insulin-like growth factor- I after Se supplementation were recorded on grazing calves (Wichtel *et al.* 1996). Florini (1985) reported that growth hormone has been shown to increase the production and secretion of IGF-1, which ultimately results in both increased muscle protein accretion and skeletal growth on calves. In addition, Dayton and White (2008) expressed that IGF-1 is needed to stimulate protein synthesis and inhibit the rate of protein degradation in muscle cells, so it facilitates the anabolic effects of GH on muscle and body growth on animals. Similarly, the results of the present calf study also support a regulatory role for circulating IGF-I in the control of calves' growth. The positive relationship between serum IGF-I and BWG suggests that knowledge of an animal's IGF-I levels at an early age may be of useful in predicting final body size.

## CONCLUSION

Selenium has a variety of functions. Owing to the antioxidant properties of glutathione peroxidase, Se effectively neutralizes free radicals. The biological role of Se-containing proteins needs to be explored in greater depth to validate positive health effects of Se on calves. The results of the present study showed that adding organic and inorganic Se to milk or colostrum had a significant effect on the performance, blood metabolites, growth hormones and antioxidant status of Holstein neonatal female calves. Knowledge of the benefit of Se in the body of neonatal calves and other species still is not sufficiently comprehensive, and a deeper study of the effects of Se may reveal a number of new biologically significant processes.

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