Effect of selenium on growth performance and blood parameters of Holstein suckling calves

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ABSTRACT

This experiment was conducted to evaluate and compare the effects of inorganic and organic selenium sources with inorganic and organic carriers on growth performance, starter feed intake, blood parameters, and the concentration of glutathione peroxidase in the blood of suckling Holstein calves. To this objective, 40 suckling Holstein calves (38.47±2.52 kg average birth weight) at 7 days of age were selected and randomly divided into four experimental groups (10 replicates). The experimental groups included control (without selenium supplement), inorganic selenium (supplemented with sodium selenate), organic selenium with inorganic carriers and, organic selenium with organic carriers. The results show that the supplementation of selenium significantly increased the glutathione peroxidase enzyme concentration (P<0.01) and can significantly reduce the concentration of plasma cholesterol (P<0.01). However, there was no significant effect of selenium supplementation on serum glucose, plasma total protein, triglyceride, and urea nitrogen concentrations. Also, none of the treatments had a significant effect on growth performance and starter feed intake.

Keywords: glutathione peroxidase, organic selenium, weight gain, calves

INTRODUCTION

Minerals are essential nutrients for livestock that are divided into micro- and macro- minerals based on the amount of animal's daily requirements. Selenium is one of the micro-minerals that has been extensively studied by animal and human nutritionists in recent years. The selenium content of plants is significantly different and depends on the condition of soil selenium. Research has shown that the soil of Iran's southern and central regions has moderate amounts of selenium, while northern regions are deficient in selenium (Nazemi et al., 2012). Therefore, it is expected that the conditions for livestock production will be improved using selenium supplements. Conventional dietary selenium supplements used by ruminants include inorganic forms such as sodium selenite (Na2SeO3·5H2O) and sodium selenate (Na2SeO4·10H2O) and organic forms, e.g., amino acid-mineral chelate forms such as selenomethionine. Chelation of minerals results in less exposure to factors that can reduce its absorption in the gastrointestinal tract and thus increase its bioavailability to the animal (Goff, 2018).

Inorganic selenium is converted by the rumen microbial population into insoluble forms with low absorption
capacity. The higher bioavailability of selenium chelate is probably due to its absorption through the active absorption mechanism of organic part (Pehrson et al., 1999; Xia et al., 2006).

Various inorganic (such as calcium carbonate) or organic (such as wheat bran) materials are used in chelated mineral products as carriers, which may be due to the different absorption and bioavailability of chelated minerals. However, to our knowledge, this effect has not yet been examined.

The selenium's biological importance as a part of the selenoenzymes structure was recognized in 1973 by the discovery of glutathione peroxidase and its role in the antioxidant defense system and cell membrane protection. Therefore, glutathione peroxidase activity is an appropriate indicator for determining the state of the body's selenium (Rotruck et al., 1973; Wang et al., 2009). Selenium supplementation has increased the activity of this enzyme in various animal species (Flohe and Brigelius, 2016). One study showed that supplementing calves with dietary chelate selenium increases the blood concentration of the glutathione peroxidase (Ebrahimi et al., 2009). Gunter et al. (2003) reported that cows fed a selenium-deficient diet had lower concentrations of glutathione peroxidase than cows fed a selenium-supplemented diet. Selenium, therefore, plays a key role in the adequate production and optimal activity of plasma lipoproteins. It reduces LDL (Low-Density Lipoprotein-Cholesterol) and triglycerides and increases HDL (High-Density Lipoprotein-Cholesterol) (Tanaka et al., 2001).

In many studies, supplementation with dietary selenium, from either organic or inorganic sources, as well as different levels of supplementation had no significant effect on weight, daily weight gain, and dry matter intake (Fokkink et al., 2009; Ebrahimi et al., 2009). In general, the recommended selenium requirement for suckling calves and all groups of dairy cows is 0.3 mg/kg dry matter (NRC, 2001). Given the role selenium plays in increasing calf resistance to disease, the use of supplements is very important because calf health guarantees future herd production performance. Also, maternal immunoglobulins cannot be transferred from the placenta to the fetus, therefore suckling calves do not have sufficient antibodies (Goff, 2018). The low number of B cells coupled with the calves’ endogenous corticosteroids and absorbed maternal hormones results in a prolonged lack of endogenous antibody response (Nagahata et al., 1991). Complement activity in newborn calves at birth is approximately 50% of that in adult cows (Firth et al., 2005).

Therefore, according to the importance of selenium in suckling calves' nutrition as well as differences in bioavailability and the function of different forms of selenium supplementation in these animals, this experiment was conducted to evaluate and compare the effects of inorganic and organic selenium sources with inorganic and organic carriers on growth performance, starter feed intake, blood parameters, and the concentration of glutathione peroxidase in the blood of suckling Holstein calves.

MATERIALS AND METHODS

Environmental conditions, calves, and management

The experiment was performed in autumn 2018, in the Bostan Agricultural and Development Company farm (Nazarabad, Alborz, Iran). The maximum, minimum, and average annual temperatures were 42 °C, -20 °C, and 14.4 °C, respectively. The average relative humidity was 53%, and the average daily wind speed was 2.2 m/s. Forty Holstein suckling calves with approximately the same initial body mass (38.47±2.52 kg, average initial body mass±SE) were separated from their dams and housed in individual pens (1.5 × 1.2 × 2.5 m, height × width × length), with a roofed section (1.5 × 1.2 m) to prevent direct sunlight or rain and a window (0.4 × 0.4 m) at the end of the pen for optimal ventilation, until weaning (56 days of age). The litter was emptied daily and after washing the pens, new disinfected and dried bagasse was replaced to maintain the comfort of the calves. The calves were fed 3 liters and 2 liters of their mother's quality colostrum (checked with a colostrometer) through pacifier buckets at the first two hours after birth and 6
hours later, respectively. This experiment was performed for 56 days (weaning) in a completely randomized design with 4 treatments and 10 replications (5 males and 5 female calves) in each experimental group. No significant health problems (diarrhea, respiratory diseases, etc.) were observed by the herd veterinarian during the experiment.

**Experimental groups and nutrition management**

According to the farm schedule, bulk milk was provided to the calves in individual steel buckets: 4 kg in two meals (at 08:00 and 17:00), from 3 to 5 days of age; 4.5 kg in three meals (at 08:00, 15:00 and 22:00), from 6 to 15 days of age; 6 kg in three meals, from 16 to 34 days of age, and 5 kg in two meals, from 35 days of age until weaning. The milk contained 3.24±0.26% fat, 2.98±0.06% protein, and 4.8±0.07% lactose.

Calves had ad libitum access to fresh water and from 3 days of age to a basal starter that was formulated according to NRC (2001) (it contained 90% concentrate and 10% wheat straw). It should be noted that the mineral supplement used in the basal starter did not contain any selenium supplement. The basal starter samples were collected monthly and after drying at 60 °C in oven for 48 hours, they were grounded with a 1 mm sieve miller and frozen at -20 °C for subsequent analysis. The samples were analyzed for dry matter, crude protein, ether extract, neutral detergent fiber, calcium, phosphorus, and selenium. The composition of the concentrate used in the basal starter feed and the nutrients contained in the basal starter are shown in Table 1.

Experimental groups included control (Control, basal starter without selenium supplement + milk without selenium supplement), inorganic selenium (InorganicSe, basal starter without selenium supplement + milk supplemented with sodium selenate), organic selenium with inorganic carriers (AvailaSe, basal starter without selenium supplement + milk supplemented with organic selenium with inorganic carriers), and organic selenium with organic carriers (ArianaSe, basal starter without selenium supplement + milk supplemented with organic selenium with organic carriers).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn grain</td>
<td>60</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>30</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>7.5</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin-mineral mixture, Selenium-free</td>
<td>1</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Chemical composition**

- Metabolizable energy (ME), Mcal/kg of DM: 2.98
- Dry matter (DM), % of concentrate: 91.2
- Crude protein (CP), % of DM: 20.4
- Ether extract (EE), % of DM: 3.2
- Natural detergent fiber (NDF), % of DM: 19.46
- Calcium, % of DM: 0.73
- Phosphorus, % of DM: 0.45
- Selenium, mg/kg of DM: 0.10

Table 1. Ingredients of the concentrate and chemical composition of basal starter on a DM basis

1 Contained per kilogram of supplement: Vitamin A: 500,000 IU, Vitamin D3: 100,000 IU, Vitamin E: 1000 IU, Calcium: 190 g, Magnesium: 19 g, Sodium: 50 g, Manganese: 2,000 mg, Ferrous: 3,000 mg, Copper: 300 mg, Zinc: 3,000 mg, Cobalt: 100 mg, Iodine: 100 mg, Antioxidant: 3,000 mg
2 Calculated based on NRC (2001)

The organic selenium with inorganic carriers was supplied from Availa®Se (Contains 2000 ppm organic selenium and carriers included calcium carbonate, silicon dioxide, etc; Zinpro Corporation, Eden Prairie, USA), and organic selenium with organic carriers supplement produced by the Ariana Biotech complementary development knowledge-based company (Contains 2000 ppm organic selenium and carriers included wheat bran, corn, soybean meal, barley, etc; Ariana Corporation, Mashhad, Iran). For selenium supplemented treatments, to ensure full intake of the daily prescribed dose by calves, as well as due to the small amount required per day, 0.3 mg/kg DM (DM starter + DM milk) selenium, according to the purity of the commercial products used, were dissolved in morning milk meal in each bucket of calves.
Data collection

Weight and height at withers of each calf were measured and recorded on the day of birth, as a covariate, and then every two weeks until the end of the experiment. The average daily weight and height increase was calculated by dividing the recorded data in each period by the number of days spent. The amount of starter feed intake was calculated by the difference between the feed offered and refused daily. At the beginning and end of the experiment, blood samples were taken from the jugular vein in two vacuum tubes (a coagulation tube and a tube containing the anticoagulant heparin) before morning meal milk. Blood samples were transferred by cold flask to the Animal Nutrition Laboratory of the Agricultural and Natural Resources College of the University of Tehran. The serum and plasma samples were separated at 3000 rpm for 15 minutes from whole blood by Cenyurion Scientific Ltd centrifuge. Whole blood samples, to determine the whole blood glutathione peroxidase activity, as well as plasma and serum samples, were kept frozen at -20 °C until parameters evaluation. Blood parameters including serum glucose, plasma cholesterol, and plasma triglycerides were measured by Glucose GOD-PAP, Cholesterol CHL-PAP, and Triglycerides GPO-PAP Kits (DIALAB Production and Testing of Chemical-Technical Products and Laboratory Instruments IZ NOE-Sued Hondastrabe, Object M55 A-2351 Wr. Neudorf Austria). Plasma total protein and serum blood urea nitrogen were measured by TP Biuret and Urea Urease-GLDH UV kits (Pars Azmoun Company, Baharestan Industrial Town, Alborz, Iran). Whole blood glutathione peroxidase concentration was measured by RANSEL kit, GTIN RS: 505 (manufactured by RANDOX UK). Each blood parameter was measured by the Nutrition Laboratory’s (Department of Animal Sciences, University of Tehran) UV-Vis spectrophotometer according to the kit provider company’s recommended protocol.

Data analysis

Repeated measure traits such as weight, height at withers, and feed intake were analyzed using the MIXED procedure of SAS software (version 9.0). The effects in the model were treatment, period, and their interactions, and if they had no significant effect they were removed from the equation. Analysis of blood parameters and glutathione peroxidase activity performed through the GLM procedure of SAS software (version 9.0).

RESULTS AND DISCUSSION

Growth performance and starter feed intake

The effect of the different selenium supplements on weight, average daily gain, height at withers, average daily height increase, and average daily starter intake are shown in Table 2. The results showed that the growth performance and average daily starter intake were not affected by selenium supplementation or the types of selenium supplements.

Consistent with the present results, Salles et al. (2014) reported that supplementation of suckling calves with 0.8 mg of organic selenium in milk or starter feed had no significant effect on weight and height at withers (Salles et al., 2014). These researchers concluded that, in suckling calves, selenium did not act as a growth promoter. Also, many studies reported that supplementation of different levels and types of selenium, such as inorganic, organic, and nano-selenium supplements, with organic or inorganic carriers, had no significant effect on growth performance in suckling calves (Nicholson et al., 1991; Fokkink et al., 2009; Ebrahimi et al., 2009; Mohri et al., 2011). In this regard, the response to selenium supplements may depend on the calves’ selenium status before and during selenium supplementation. In addition, studies on pregnant cows did not show any significant effect of selenium supplements on birth weight and weight gain of their calves (Gunter et al., 2003; Davis et al., 2005).

Evidence suggests that the role of selenium in growth is important because it can reduce the concentration of pituitary growth hormone when the concentration of triiodothyronine decreases. Peripheral growth hormone concentrations do not change in selenium deficiency, and this proves that selenium can alter somatotropic function by regulating IGF-I and IGF-II production and secretion, the number of somatotropic receptors, or peripheral IGF (Insulin-like Growth Factor) binding...
Table 2. The effect of the different selenium supplements on growth performance and starter intake

<table>
<thead>
<tr>
<th>Item</th>
<th>control</th>
<th>InorganicSe</th>
<th>AvaiaSe</th>
<th>ArianaSe</th>
<th>SEM</th>
<th>T</th>
<th>P</th>
<th>T×P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final weight (kg)</td>
<td>68.85</td>
<td>69.15</td>
<td>69.75</td>
<td>70.00</td>
<td>0.40</td>
<td>0.51</td>
<td>&lt;0.001</td>
<td>0.89</td>
</tr>
<tr>
<td>Average daily gain (kg)</td>
<td>0.68</td>
<td>0.69</td>
<td>0.69</td>
<td>0.71</td>
<td>0.01</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td>0.94</td>
</tr>
<tr>
<td>Final height at withers (cm)</td>
<td>84.75</td>
<td>85.40</td>
<td>85.45</td>
<td>85.88</td>
<td>0.25</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td>0.60</td>
</tr>
<tr>
<td>Average daily height increase (cm)</td>
<td>0.21</td>
<td>0.21</td>
<td>0.20</td>
<td>0.21</td>
<td>0.006</td>
<td>0.86</td>
<td>&lt;0.001</td>
<td>0.48</td>
</tr>
<tr>
<td>Average daily starter intake (kg)</td>
<td>0.78</td>
<td>0.80</td>
<td>0.80</td>
<td>0.79</td>
<td>0.11</td>
<td>0.52</td>
<td>&lt;0.001</td>
<td>0.87</td>
</tr>
</tbody>
</table>

* Means within a row with different superscripts are significantly different (*P*<0.05)
1 *P*-values include: T: Treatment, P: Period, T×P: Treatment × Period
2 Treatments include: control: without selenium supplement, InorganicSe: Supplemented with sodium selenate, AvaiaSe: Supplemented with organic selenium with inorganic carriers, ArianaSe: Supplemented with organic selenium with organic carriers

proteins concentration (Wichtel et al., 1996). However, the 5-Iodothyronine deiodinase enzyme is one of the last proteins to be affected by selenium deficiency (Mehdi and Dufrasne, 2016). This may explain why several studies have observed that selenium supplementation has no significant effect on weight gain and growth performance.

The results showed, consistent with other studies, the different sources of selenium supplement, including organic or chelated form and with organic or inorganic carriers, have no significant effect on dry matter intake (Nicholson et al., 1991; Gunter et al., 2003; Fokkink et al., 2009).

**Blood parameters and glutathione peroxidase enzyme**

The results of the blood parameters analysis are shown in Table 3. According to the results, different sources of organic and inorganic supplements of selenium with inorganic or organic carriers had no significant effect on serum glucose and plasma triglyceride, protein, and urea nitrogen concentrations.

In many studies, the use of different sources of selenium supplements, i.e. with inorganic or organic carriers, had no significant effect on glucose, urea nitrogen, and total protein concentrations (Shinde et al., 2009; Mohri et al., 2011). However, Ebrahimi et al. (2009) reported that due to the insulin-like effect of selenium, the use of selenium supplements reduces serum glucose levels in one-month-old suckling calves. This discrepancy can be due to differences in the amount of selenium in the basal diet or the age of the calves.

Analysis of blood parameters (Table 3) showed that supplementation of selenium significantly reduced the plasma cholesterol concentration of suckling calves (*P*<0.01). Also, these results showed a significant difference between the groups receiving organic and inorganic selenium supplements. Organic selenium treatments had lower cholesterol levels than the inorganic selenium group (*P*<0.05). In addition, among the two organic selenium groups, organic selenium with inorganic carriers had significantly lower cholesterol concentrations than organic selenium with organic carriers (*P*<0.05). In some studies have been reported that plasma cholesterol concentration decreased in calves and lambs by selenium supplementation (Ebrahimi et al., 2009). Qu et al. (2000) found that selenium deficiency in mice due to increased activity of the enzyme HMG-CoA (β-Hydroxy β-methylglutaryl-CoA) reductase, an enzyme that regulates cholesterol biosynthesis in mammals, increased plasma cholesterol and LDL concentrations. Selenium is an essential factor for the expression of genes involved in the synthesis of apolipoprotein B and the enzyme HMG-CoA reductase. Selenium supplementation can decrease mRNA expression responsible for the production of this enzyme.
<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment1</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>InorganicSe</td>
<td>AvailaSe</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.731</td>
<td>5.686</td>
<td>5.687</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.281</td>
<td>0.280</td>
<td>0.282</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.641*</td>
<td>3.358a</td>
<td>2.804d</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>60.70</td>
<td>60.50</td>
<td>60.90</td>
</tr>
<tr>
<td>Urea nitrogen (mmol/L)</td>
<td>5.018</td>
<td>4.904</td>
<td>4.714</td>
</tr>
<tr>
<td>Glutathione peroxidase (Unit/L)</td>
<td>27595.83c</td>
<td>32632.96a</td>
<td>36698.90c</td>
</tr>
</tbody>
</table>

* Means within a row with different superscripts are significantly different (P<0.05)

1 Treatments include: control: without selenium supplement, InorganicSe: Supplemented with sodium selenate, AvailaSe: Supplemented with organic selenium with inorganic carriers, ArianaSe: Supplemented with organic selenium with organic carriers.

Based on the results, the use of various forms of selenium supplements, especially organic selenium supplements, leads to a significant increase in the concentration of glutathione peroxidase enzyme (P<0.01). However, there was no significant difference in glutathione peroxidase enzyme concentration between organic selenium with inorganic carriers and organic selenium with organic carriers. These results are consistent with studies that have shown the supplementation, form, and amount of selenium supplements affect the concentration of glutathione peroxidase enzyme. These researchers also reported that organic and nano-selenium forms have the most effect on increasing the concentration of this enzyme (Knowles et al., 1999; Yue et al., 2009; Ebrahimi et al., 2009).

When selenium is available, GSH-Px (Glutathione peroxidase) concentrations increase and can compensate for stressful conditions (Flohe and Brigelius, 2016). The reason for the difference between various sources of selenium supplements in terms of the ability to increase the concentration of the glutathione peroxide enzyme is probably due to the absorption pathways of selenium in various forms. The mechanism of intestinal absorption of inorganic selenium is diffusion and its absorption efficiency is less than 50% (Holben et al., 2002). Nevertheless, the organic selenium is absorbed in the intestine by an active transport mechanism and is distributed non-specifically in all proteins at the location of the methionine amino acid during protein synthesis, which can produce a rich and reversible source of selenium in tissues and organs (Schrauzer, 2000).

**CONCLUSIONS**

The aim of the present study was to compare the effects of inorganic and organic selenium sources with inorganic or organic carriers on growth performance, feed intake, blood parameters, and glutathione peroxidase concentration in the blood of suckling Holstein calves. Results indicated that the supplementation of selenium increases the glutathione peroxidase enzyme concentration. Based on our results daily supplementation of 0.3 mg of selenium supplements into suckling calves’ milk can reduce the concentration of their plasma cholesterol. However, selenium supplementation had no significant effect on serum glucose, plasma total protein, triglyceride, and urea nitrogen concentrations. Also, none of the treatments had a significant effect on growth performance and starter feed intake.

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