**The effect of feeding inorganic and organic selenium sources on the performance and content of selenium in milk of transition dairy cows.**

**Short title:** inorganic and organic selenium sources …

**Abstract**

The aim of this study was to determine the effects of supplementing close-up and fresh dairy cow’s diets with sodium selenite or organic sources of selenium (Se) on the dry matter intake (DMI), milk production and composition. Twenty-four multiparous Holstein dairy cows were balanced by body condition score (BCS), previous lactation milk yield and expected calving date and randomly assigned to 1 of 4 experimental treatments. Treatments were: control (basal diet without Se supplementation with 0.10 and 0.15 mg of Se/ kg DM in close-up and fresh diets, respectively), sodium selenite supplementation (0.50 mg of Se/kg DM; Se-S), selenium yeast supplementation (0.50 mg of Se/kg DM; Se-Y) and selenomethionine supplementation (0.50 mg of Se/kg DM; Se-M). Neither Se supplementation nor Se source affected DMI pre- and postpartum. Milk production and composition were not different across se sources, but concentrations of Se in milk were 14 and 10% greater for cows fed Se-M and Se-Y than for those fed control, respectively. Cows fed Se–M had lower somatic cell count than control (SCC; 173.11 vs. 318.89 cells × 103 /mL). The changes of BW and BCS pre- and postpartum were not affected by treatments over experimental period. The results of present study showed that selenomethionine supplementation compared to other sources decreased SCC in transition cows. Milk Se concentrations increased in Se-Y and Se-M more effectively than Se-S indicating selenomethionine could replace Se-S as an effective organic Se source for transition dairy cows.

**Keywords:** inorganic selenium, organic selenium, performance, transition period.

**Introduction**

During early lactation, a large increase in nutrient requirement occurs to support lactation resulting in negative nutrient balance. Dairy cows often experiences a declined antioxidant capacity and therefore an increased oxidative stress associating with more produce of reactive oxygen species (**ROS**; Sordillo and Aitken, 2009; Gong and Xiao, 2016). Over generation and repletion of ROS exposures transition cows to metabolic disorders and infectious diseases (Gong and Xiao, 2016). Thus, promoting antioxidant status could reduce consequences of oxidative stress and improve health and productive performance during the transition period.

The pivotal role of selenium (Se) as a trace element to maintain antioxidant status in humans and animals has been well known and it has been often supplemented in the diet of animals (Schwarz and Moltz, 1957). The diets of dairy cows could be supplemented with selenium in inorganic (sodium selenite and sodium selenate) or organic forms (Se yeast and Se methionine). Inorganic forms of Se are widely supplemented in diet due to their low prices. Whereas, relative to inorganic Se, organic Se is absorbed with a higher rate, and has a greater biological activity, a higher accumulation rate in tissue, and lower toxicity (Ortman and Pehrson, 1999; Boldizarova et al., 2005; Briens et al., 2014).

The metabolisms of organic and inorganic forms of Se are different in the body (Calamari et al., 2010). Some studies reported supplementing of selenium yeast (**SY**) increased whole blood and milk Se concentrations (Slavik et al., 2008), improved antioxidant status (Gong et al., 2014), and promoted the Se status than sodium selenite (Doucha et al., 2009). Likewise, Calamari et al. (2010) reported that a greater milk Se concentrations in cows fed SY than sodium selenite.

Sun et al. (2017) reported supplementing hydroxyselenomethionine in mid lactation diets for 10 wks did not affect DMI, milk yield and composition, or blood biochemical parameters compared to those supplemented selenite selenium. However, they observed that cows fed hydroxyselenomethionine had higher serum activity of glutathione peroxidase, total antioxidant capacity, superoxide dismutase and greater total Se in milk and plasma than the selenite selenium group.

We hypothesized that cows fed organic sources of selenium during pre and postpartum would have higher serum Se concentrations and consequently have improved antioxidant indices, health and production performance than cows fed selenite. Therefore, aim of this study was to investigate the effects of selenomethionine, selenium yeast and sodium selenium supplementation on dry matter intake, body weight and body condition score changes, milk production and composition and the selenium concentrations in milk over the transition period.

**Materials and methods**

**Feeding, experimental design and management of cows**

The experiment was carried out on a commercial dairy herd in Iran (FKA Animal Husbandry and Agriculture Co., Isfahan, Iran) from May to July 2016. Twenty-four multiparous dairy Holstein cows were randomly assigned in a balanced manner to 1 of 4 experimental treatments based on BCS, previous lactation milk yield and expected calving date from 21 d before expected calving date to 21 days in milk (DIM). At enrollment, cows averaged 820 ± 65.25 kg of BW, 3.79 ± 0.38 of BCS, and 14585.70 ± 1196.90 kg of 305-d mature equivalent milk.

Animals were housed in individual 4 × 4 m2 stalls bedded with sand with free access to water from 20± 5 d before calving to 21 DIM. During the close-up and after calving period, cows received basal isoenergetic and isonitrogenic diets with 0.1 and 0.15 mg of Se /kg DM, respectively (Table 1) for ad libitum intake thrice daily at 0800, 1600, and 2400 h. The diets offered to close-up and fresh cows (Table 1) were formulated according to the NRC (2001) model. After calving, experimental cows were the first group to be milked at each shift at 0700, 1500, and 2300 h. Sodium selenite as inorganic source and Se-enriched yeast ([Biorigin., Brazil - Selemax](https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&cad=rja&uact=8&ved=0ahUKEwiWm9nlhdTUAhVGaVAKHYD6ATEQFggxMAI&url=http%3A%2F%2Fwww.biorigin.net%2Fbiorigin%2Findex.php%2Fen%2Fsolucoes-2%2Fnutricao-animal-2%2Fnutri-especies-2%2Fprodutos-2%2Fselemax-aves-2&usg=AFQjCNF125nWtrnq8np44R7r9kEgV_zADg) 2000 ppm) and Se-methionine (Arkop., Poland - Amino Selstar 2000 ppm) as inorganic source were supplemented into close- up and fresh diets to provide 0.50 mg of supplemental Se/kg, respectively. After calving, cows received the same sources and concentration of supplemental Se as those fed during the close- up period. Treatments were: control (basal diet without Se supplementation with 0.1 and 0.15 mg of Se/ kg DM in close-up and fresh diets, respectively; CO), sodium selenite supplementation (0.5 mg of Se/kg of DM; Se-S), Se yeast supplementation (0.5 mg of Se/kg of DM; Se-Y) and selenomethionine supplementation (0.5 mg of Se/kg of DM; Se-M).

**Diet composition**

Ingredients and nutrient chemical composition of diets are presented in Table 1. The close-up and fresh diets were formulated to meet NRC (2001) requirements for 1.60 and 1.70 Mcal NEL , 13.70 and 16.10% CP, and 34.60 to 32.20% NDF per kg DM, respectively. To provide 0.30 and 0.35 mg of Se /kg DM in excess NRC (2001) recommendation, close-up and fresh diets were supplemented by different sources of Se.

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| Table 1. Feed ingredients and chemical composition of the diets fed during close-up, and fresh periods (% of DM) | | |
|  | Basal diets | |
| Ingredient | Close-up | Fresh |
| Legume forage hay, mature | 12.4 | 17.04 |
| Corn silage, normal | 47.5 | 21.31 |
| Sugar beet pulp | 0 | 9.47 |
| Barley grain, ground, dry | 10.08 | 11.89 |
| Corn grain, ground, dry | 11.49 | 14.5 |
| Soybean meal, solvent | 1.94 | 8.14 |
| Canola meal, mechanical extraction | 5.42 | 0 |
| Extruded full-fat soybean | 1.16 | 2.58 |
| Fish meal | 1.55 | 1.72 |
| Cottonseed, whole with lint | 2.39 | 8.81 |
| Corn gluten meal | 1.55 | 1.58 |
| Mineral premix1 | 0.38 | 0.25 |
| Vitamin premix2 | 0.38 | 0.23 |
| Magnesium oxide | 0.29 | 0.23 |
| Calcium carbonate | 1.36 | 0.19 |
| Dicalcium phosphate | 0 | 0.33 |
| potassium carbonate | 0 | 0.19 |
| NaHCO3 | 0 | 0.57 |
| Calcium chloride | 0.58 | 0 |
| Magnesium oxide | 0.69 | 0 |
| Biotin | 0.004 | 0.004 |
| Choline chloride | 0.37 | 0.24 |
| Niacin | 0.04 | 0.05 |
| Avila 4 | 0.06 | 0.03 |
| Monensin | 0.01 | 0.01 |
| Live yeast | 0.004 | 0.003 |
| Bentonite | 0.352 | 0.393 |
| Salt | 0 | 0.24 |
| Chemical composition |  |  |
| Dry Matter (%) | 41 | 59 |
| NEL (Mcal/kg DM) 3 | 1.6 | 1.7 |
| Protein (%) | 13.7 | 16.1 |
| NDF (%) | 34.6 | 32.2 |
| Ca (%) | 1.31 | 0.94 |
| P (%) | 0.39 | 0.43 |
| DCAD (meq/kg) 3 | -57 | 288 |
| Se (ppm)4 | 0.1 | 0.15 |
| 1 Premix contained 50 g of Ca/kg, 11 g of Mg/kg, 15 g of Zn/kg, 3 g of Cu/kg, 0.15 g of I/kg, 0.05 g of Co/kg.  2 Premix contained 1800000 IU of vitamin A/kg, 200000 IU of vitamin D/kg, 15000 IU of vitamin E/kg and 1.25 g of butylated hydroxytoluene/kg as a synthetic antioxidant.  3 Estimated from NRC (2001).  4 Selenium were measured by an Inductively Coupled Plasma-Mass Spectrometry. | | |

**Sampling and data collection**

Dry matter intake was individually determined from −21 d relative to expected calving until 21 d after parturition. Samples of TMR and orts were weekly taken for DM measurement, and were dried at 60°C for 48 h, and then composited by treatment. Dried pooled samples of TMR diets and refusal were ground through a 1-mm screen in a Wiley Mill and analyzed for analytical DM (AOAC, 1990; method 930.15), CP by the Kjeldahl method (AOAC, 1990; method 984.13), ether extract by the Soxhlet extraction method with diethyl ether (AOAC, 1990; method 920.39), Ash (ignition at 600°C for 2 h; AOAC, 1990, method 942.05), and ADF by the cetyl-trimethyl-ammonium bromide H2SO4 (CTAB) and 1N method (AOAC, 1990; method 973.18). The NDF content was determined by heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991). The amounts of Se concentrations in diets and milk were measured by an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) device (using a Varian Model SpectrAA 220 atomic absorption spectrometer, Mulgrave, Victoria, Australia).

Cows were weighed at -21, 0, and 21 d relative to calving, and BW loss due to calving was calculated as after calving weight (calving day) minus the BW at the beginning of the experiment (-21 d). The changes of BW from 1 to 21DIM were calculated as BW at the end of experiment (21d) minus BW at the end of experiment. Cows were scored for body condition using a 5-point scale with 0.25 increments to determine BCS changes at calving and at the end of experiment (Wildman et al., 1982) by 2 skilled evaluators at -21, 0, and +21 d relative to calving. Results from the two evaluators were averaged and used for analysis.

Milk yield was recorded over 21 DIM and milk samples were taken from 3 consecutive milking’s and composited proportionally to milk yield in 100 mL sterile tubes twice a week. Immediately after collection, milk samples were analyzed to determine milk composition (fat, protein, lactose), milk urea nitrogen (MUN) and SCC using a MilkoScan Minor (CombiFoss 78110; Foss Analytical A/S, Hillerød, Denmark). The rectal temperature was measured by a thermometer during the first 10 d after calving.

**Statistical analysis**

The data were analyzed by PROC MIXED of SAS (version 9.3; SAS Institute Inc., Cary, NC). Dry matter intake, milk yield and composition (fat, protein, lactose, MUN and SCC), serum biochemical parameters, and antioxidant indices data (pre and postpartum) were analyzed as repeated measures and Time (DIM and week) was included in the model as a repeated variable. Based on the lowest Akaike information criterion, corrected Akaike information criterion, and Bayesian information criterion values for each variable analyzed the most suitable covariance structure were used (Littell et al., 1998). The following model was used: Y ijk = μ + Ti + Timej + (T × Time)ij + Cow(i)k + eijk,

where Yijk is the dependent variable, μ is the overall mean, Ti is the fixed effect of treatment, Timej is the fixed effect of sampling time, (T × Time)ij is fixed interaction between treatment and sampling time, Cow(i)k is random effect of cow nested within treatment, and eijk is the error term. The same model was used to analyze BCS and BW changes pre- and postpartum, but the fixed effects of time and their interactions were removed from the ANOVA model. Previous lactation yield, BCS, BW and the concentrations of serum metabolites and antioxidant indices obtained at −21 d relative to expected calving date were used as covariates and covariates were excluded from the model if they were not significant (*P* > 0.1). Data are reported as LSM and statistical significances were indicated at *P* ≤ 0.05 and 0.05 < *P* < 0.10 as trends toward significance using the Tukey’s multiple comparison test.

**Result and discussion**

**Close-up period**

**Dry matter intake, body weight and body condition score changes.** Effects of supplementation and source of Se on DMI, BW and BCS changes prepartum are presented in Table 2. Neither Se supplementation nor Se source did not affect DMI, BW and BCS changes. Similar to the results of present study, Weiss and Hogan (2005) did not observe any effect of supplementing different sources of Se during close-up period on DMI and BW changes. Likewise, Muegge et al., (2016) did not observe any difference in DMI, BW, and BCS in beef cows supplementing with different sources of Se during the last 80 d of gestation and the first 108 d of lactation.

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| Table 2. The effect of supplementing inorganic and organic selenium sources on dry matter intake, BW and BCS changes prepartum. | | | | | | | | |
| *P*- values | | |  | Experimental treatments1 | | | |  |
| Time  ×  Treatment | Time | Treatment | SEM | Se-M | Se-Y | Se-S | CO | Items |
| 0.97 | 0.7 | 0.72 | 1.06 | 11.59 | 11.92 | 11.08 | 10.29 | DMI (kg/d) |
| - | - | 0.90 | 39.63 | 860.67 | 790.00 | 816.67 | 814.17 | Initial BW |
| - | - | 0.29 | 29.96 | 54.16 - | 52.50- | 52.30- | -57.50 | BW changes2 |
| - | - | 0.80 | 0.23 | 3.75 | 3.91 | 3.70 | 3.79 | Initial BCS. |
| - | - | 0.30 | 0.2 | 0.14- | 0.18- | 0.36 - | 0.64- | BCS changes2 |
| a-b Means in the same column with no common superscripts are significantly different (P<0.05).  1 Treatments were: control (basal diet without Se supplementation with 0.1 and 0.15 mg of Se/ kg DM in close- up and fresh diets, respectively), sodium selenite supplementation (0.5 mg of Se/kg of DM; Se-S), selenium yeast supplementation (.0.5 mg of Se/kg of DM; Se-Y) and selenomethionine supplementation (0.5 mg of Se/kg of DM; Se-M).  2 The difference between the BW and BCS before and after calving. | | | | | | | | |

**Postpartum period**

**Dry matter intake, body weight and body condition score changes.** Effects of supplementation and source of Se on DMI, BW and BCS changes postpartum are showed in Table 3. Dry matter intake, BW and BCS changes postpartum were not different across experimental treatments (*P* > 0.05). Weiss and Hogan (2005) found no effect of supplementing different sources of se on DMI during the transition period. They reported a higher BW for supplemented cows with selenite compared to yeast Se which might be due to differences in BW at enrollment. In according to the present study results, others studies investigating the effects of dietary Se source in lactating cows did not observe any change in DMI (Heard et al., 2007; Ran et al., 2010; Salman et al., 2013; Sun et al., 2017).

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| Table 3. The effect of supplementing inorganic and organic selenium sources on dry matter intake, BW and BCS changes, milk production and composition, and milk Se postpartum. | | | | | | | | |
| P-Value | | | SEM | Experimental Treatments1 | | | |  |
| Time  ×  Treatment | Time | Treatment | Se-M | Se-Y | Se-S | CO | Items |
| 0.66 | 0.06 | 0.80 | 1.72 | 16.79 | 15.54 | 14.91 | 15.90 | DMI (kg/d) |
| - | - | 0.77 | 14.36 | 53.34- | 38.33- | 46.32- | -48.33 | BW changes2 |
| - | - | 0.96 | 0.32 | 0.48- | 0.51- | 0.31- | 0.31- | BCS changes2 |
| 0.23 | <0.01 | 0.49 | 2.24 | 34.86 | 34.07 | 32.93 | 31.85 | Milk yield (kg/d) |
| 0.18 | 0.08 | 0.66 | 0.25 | 3.90 | 3.80 | 3.54 | 3.93 | Milk fat (%) |
| 0.77 | <0.01 | 0.80 | 0.14 | 3.28 | 3.21 | 3.16 | 3.15 | Milk protein (%) |
| 0.63 | 0.41 | 0.96 | 0.18 | 4.81 | 4.82 | 4.75 | 4.84 | Lactose (%) |
| 0.27 | 0.56 | 0.03 | 31.58 | 173.11 b | 245.83 ab | 250.71ab | 318.89 a | SCC (103/dl) |
| 0.19 | 0.84 | 0.01 | 0.32 | 12.66 a | 12.15 a | 11.61ab | 11.04 b | Se (mg/l) |
| <0.01 | 0.41 | 0.90 | 1.83 | 33.74 | 33.30 | 30.77 | 30.84 | FCM %4 (kg/d) |
| 0.14 | <0.01 | 0.52 | 0.13 | 1.32 | 1.30 | 1.16 | 1.21 | Fat (kg/d) |
| 0.44 | <0.01 | 0.37 | 0.08 | 1.13 | 1.08 | 1.03 | 0.98 | Protein (kg/d) |
| 0.42 | <0.01 | 0.70 | 0.11 | 1.66 | 1.64 | 1.57 | 1.53 | Lactose (kg/d) |
| a-b Means in the same column with no common superscripts are significantly different (P<0.05).  1 Treatments were: control (basal diet without Se supplementation with 0.1 and 0.15 mg of Se/ kg DM in close- up and fresh diets, respectively), sodium selenite supplementation (0.5 mg of Se/kg of DM; Se-S), selenium yeast supplementation (.0.5 mg of Se/kg of DM; Se-Y) and selenomethionine supplementation (0.5 mg of Se/kg of DM; Se-M).  2 Changes in BW and BCS from calving to 21 DIM. | | | | | | | | |

**Milk production and composition.** The results of Se supplementation with different source on milk production and composition are presented in Table 3. According to DMI results, milk production was affected by neither Se source nor its levels of supplementation. However, milk production was not affected by experimental treatments, relative to control, cows in selenite, Se-yeast and Se-Met produced 1.08, 2.22 and 3.01 kg more milk. There was not any effect of supplementing Se with different form on milk composition (P >0.05; Table 3).

In agreement with our results, Weiss and Hogan (2005) observed no effect of supplementing Se yeast compared with selenite on milk yield and composition during the peripartum. Likewise, Ceballos et al., (2010) found no effects of prepartum Se supplementation and type of supplementation on milk yield in pasteurized heifer. Calamari et al. (2010) reported that milk yield and composition were affected by neither the source of Se (selenite or Se yeast) nor Se level of supplementation (0.31 or 0.50 mg of Se/kg DM) when dairy cows consumed the same basal diet containing 0.10 mg of Se/kg DM. Likewise, Sun et al. (2017) found no effect of Se sources (hydroxyselenomethionine and sodium selenite) and Se supplementation (0.1, 0.3, or 0.5 mg of se/kg DM) compared to a basal diet containing 0.06 mg of Se/kg DM in mid lactation cows. Additionally, milk yield and composition in the present study were supported by other (Juniper et al., 2006; Heard et al., 2007; Ran et al., 2010; Salman et al., 2013; Calamari et al., 2010; Gong et al., 2014) reporting dietary Se source did not markedly affect milk yield or composition in lactating cows.

The somatic cell count (SCC) was affected by Se supplementation and Se sources (*P*= 0.03), so that relative to control, cows in Se- Met had lower SCC (173.11 vs. 318.89 103 cell/mL; *P* <0.01) and cows in Se –yeast tended to have lower SCC (*P*= 0.10) while supplementing of dietary Selenium as selenite did not affect milk SCC (*P* > 0.05).

Some studies (Rowntree et al., 2004; Xu et al., 2007) reported that sufficient supply of dietary Se improved the antioxidant status in dairy cows using increase in the activity of GPX in whole blood, erythrocyte and serum and decline in the serum malondialdehyde concentration. In addition, Hogan et al. (1990) reported that polymorphonuclear neutrophils (**PMN**) isolated from Se-supplemented cows had greater intracellular killing of *S. aureus* than unsupplemental cows. Miranda et al. (2011) with supplementing Se to bovine mammary cells in vitro reported that Se has an important role in the improvement of udder health through improved antioxidant status. Likewise, It has been reported supplementing sufficient content of Se in the diets of dairy cows reduced SCC (Silvestre et al., 2007) and the incidence of mastitis of dairy cows and improved the immune status and mammary health (Weiss, 2003; Mukherjee, 2008). Although, Calamari et al. (2010) and Juniper et al, (2006) observed no effects of sources and supplementation of Se on milk SCC of late and early lactation cows.

**Milk selenium concentrations postpartum.** The effects of dietary Se supplementation with inorganic and organic forms on total Se concentrations in milk are presented in Tables 3.

There was an effect of Se supplementation and source on milk Se concentration (*P* = 0.01), so cows in Se-Met (12.66 mg/L) and Se-Y (12.15 mg/L) had higher milk Se concentration compared to control (*P* < 0.05), but this effect was not observed in Se-S cows.

Weiss and Hogan (2005) observed that Se yeast supplementation increased up to 2 fold colostrum and milk concentrations of Se compared to selenite supplementation during the dry period and early lactation. In the present study, increased milk Se concentrations showed that Se-Met and Se- Y are more effectively improved milk Se than and selenite. The results of this study were supported others reporting Se supplementation using organic sources of Se increased milk Se concentrations in lactating dairy cows (Weiss, 2005; Juniper et al., 2006; Phipps et al.,2008; Calamari et al.,2010). In the present study, cows in Se-Met and Se yeast had an increase 14 and 10 % in milk Se relative to control while Sun et al. (2017) and Ran et al. (2010) reported an increase 53 and 125% in milk Se concentrations with supplementing Se as Se-yeast and hydroxyselenomethionine in early and mid-lactation cows compared to selenite. Differences in magnitude of responses might be because of variations in duration and level of supplementation of dietary Se, DIM and Se level in basal diet (control).

Methionine was uptaken for synthesis milk protein by the mammary gland (Weiss, 2003). Selenium in organic sources can be included in milk proteins along with Met uptake as SeMet. Thus, increases in the Se milk found in cows supplementing Se yeast and Se-Met might be due to the unremitting Met uptake in the milk protein.

In general, neither Se supplementation nor Se sources did not affect DMI, productive performance, BW and BCS changes and antioxidant indices over the transition period. Decreased SCC and increased albumin in Se-M and Se-Y cows could suggest an improve in immune status.

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