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The Effect of Injection of Different Levels of Selenium and Vitamin E in Late Pregnancy of Cows on Performance, Thyroid Hormones, some Blood Metabolites and Skeletal Growth Indices of Their Calves

Research Article

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ABSTRACT

In order to evaluate of injection of different levels of vitamin E-selenium supplementation in late pregnancy of cows and its effects on growth performance and immunity of their male calves, 28 Holstein dairy cows 28 days before calving in a complete design were randomly selected with 7 replications and 4 treatments. Experimental treatments included injection of 0, 15, 30 and 45 mL of E-selenium supplementation before calving. Injections of selenium (sodium selenite) and vitamin E (DL alpha tocopherol acetate) supplements were given at 28 and 14 days before delivery. Blood samples were taken from calves at the end of the experimental period (45 days after birth) to measure blood parameters. The results showed that injection of vitamin E-selenium solution in pregnant cows did not affect birth weight, feed intake and feed conversion ratio and dry matter digestibility of calves, while the final weight of calves increased (P<0.05). Blood parameters of glucose, albumin, cholesterol, globulin and urea were not significantly affected, but injection of vitamin E-selenium in pregnant cows increased blood triglyceride concentration compared to the control group (P<0.05). The results showed that the glutathione peroxidase concentration in calves was significantly increased by injecting vitamin E and selenium solution in pregnant cows (P<0.05). Also, triiodotronine and tetraidotronin hormones concentration was significantly increased by injecting 15 mL of vitamin and selenium solution in pregnant cows compared to the control group. However, betahydroxybutyrate, malondialdehyde and non-esterified fatty acids concentrations were not affected by solution injection. The results showed that skeletal growth of calves was not affected by injection of vitamin E-selenium solution in dry cows. In general, it is concluded that injection of vitamin E-selenium in pregnant cows could improve the final weight, glutathione peroxidase concentration, the hormones triiodotronine and tetraiodotronine in calves.

KEY WORDS immunity, newborn calf, selenium, transmission period, vitamin E.

INTRODUCTION

The gross effects of selenium and vitamin E deficiency on animal production and the potential benefits of supplementation are recognized, and the influence of these micronutrients on the resistance of animals to a variety of infections has also been reported. Selenium (Se) and vitamin E (VE) are essential micronutrients that share a common biological role as antioxidants (Suttle, 2010). Selenium (Se) is an essential trace mineral in animals that is required to maintain normal physiological functions and provides a significant dietary source of antioxidant defenses (Sordillo, 2013). It is obtained by animals as components of the diet and its transplacental transfer is an important factor for their offspring (Moeini et al. 2009). Selenium deficiencies play a role in numerous economically important livestock diseases, problems that include impaired fertility, abortion, retained placenta, and neonatal weakness (Mohri et al. 2011). Most diseases in dairy cows occur at or just after calving, which is a period associated with immune suppression, resulting in an increased susceptibility to infections (Antunović et al. 2014; Abdolmaleki et al. 2017). Selenium and vitamin E work together as a team to prevent lipid peroxidation. Vitamin E quenches lipid hydroperoxyl radicals and the resulting lipid hydroperoxide is then converted to the lipid alcohol by selenocysteine-containing glutathione peroxidase (Nayyar and Jindal, 2010). The Se and vitamin E (α tocopherol) status of dairy cows is one important component of a well functioning immune system because of its antioxidant effects on cows and young dairy calves (Nayyar and Jindal, 2010). At parturition, plasma concentrations of vitamin E were found to decrease by 47 percent, because of secretion of the vitamin into the udder during colostrogenesis, decreased dry matter intake (DMI) at calving, and an increased need for antioxidants during this time (Hefnawy et al. 2014). Passive transfer of colostral immunoglobulins is vitalto short-term health and survival of neonates, and limited data suggest that inadequate transfer occurs in 10 to 25 percent of newborn beef calves (Kafilzadeh et al. 2014). Low serum immunoglobulins in young calves were related to increased incidence of disease. Previous investigations have revealed that serum IgG concentrations decreased at parturition (Sushma et al. 2015).

The amount of nutrients transferred to the offspring depends on maternal nutrient status and the efficiency of the transplacental and mammary transport mechanisms. The passage of trace elements and other nutrients through the placenta is required for fetal nutrition and growth as well as for maternal and fetal physiological functions during gestation. Provision of Se to the mother during pregnancy is an effective method to meet Se requirements in the newborn, as it efficiently passes through the placental barrier into fetal tissues and is also transferred into colostrum and milk (Rock et al. 2001; Stewart et al. 2012). A close relationship has been detected between offspring serum Se and maternal levels after feeding with Se, once the offspring obtains this nutrient via the placenta before birth (Hefnawy et al. 2014). The serum or plasma Se concentrations provide a good indication of Se status in ruminants (Asadi et al. 2021a).

Administration of Se and VE in sows during the late stage of pregnancy affects colostral immunoglobulin concentrations and passive immunity. It has also been reported that an adequate supply of Se alone or in combination with VE, in cows at late pregnancy, increased colostral and post-suckle serum IgG concentrations. Dietary deficiencies of Se decrease IgG and IgM concentrations in plasma (Balicka-Ramisz and Jastrzębski, 2014). A divergence of opinion regarding the effects of Se on hematological indicators was often presented in literature. A positive effect of Se supplement on hematological indicators was observed by several authors (Antunović et al. 2014; Abdolmaleki et al. 2017), but not confirmed by others (Shi et al. 2011; Asadi et al. 2018). Most of the available information in this field is based on studies outside the Middle East, where the conditions for dairy cows are different, for example, in housing systems, feeding, climate, and management. In addition, soils in many of the areas of Iran are Se deficient, and feedstuffs grown on these soils will not provide adequate dietary Se (Aliarabi and Fadayfar, 2016). The objective of this study was to determine the effect of injection of different levels of selenium and vitamin E in late pregnancy of cows on performance, thyroid hormones, some blood metabolites and skeletal growth indices of their calves.

MATERIALS AND METHODS

Location, diets, animals and experimental design

This experiment was conducted in Mazandaran province and Behshahr city in 2021 and the relevant experiments were carried out in the Gorgan University of Agricultural Sciences and Natural Resources (Gorgan, Iran) accordance with the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010). For this purpose, 28 dairy cows were selected based on calving cycle, body weight and body condition score and were divided into 4 groups and 7 repetitions. The experimental treatments included control and injection of 15, 30 and 45 ml of selenium and vitamin E supplements in pre-calving cows, selenium (sodium selenite) and vitamin E (DL-alpha tocopheryl acetate) supplements were injected at 28 and 14 days before calving was performed. Each milliliter of selenium and vitamin E injectable supplement contained 50 mg of vitamin E in the form of tocopherol acetate and 0.5 mg of selenium in the form of sodium selenite. After birth, male calves born from each group were examined for 45 days. In the first 24 hours after birth, the calves were separated from their mothers and the navels were disinfected with tincture solution and after weighing, they were transferred to individual boxes. Then 4 liters of colostrum were fed in two times and in the first 8 hours of birth, and colostrum feeding continued for another 2 days based on 10% of body weight. The male calves were fed milk twice a day (8:30 a.m. and 4:30 p.m.). On the 4th day of birth, the calves were transferred to individual concrete boxes in the calving area. Rations were adjusted based on NRC (2001). Calves were given drinking water freely from the 5th day of birth, and only one hour before to one hour after milking, the calves were prevented from having access to water. The amount of 10% of dry alfalfa was added to the starter diet of the calves from the 20th day after birth in the form of chopped pieces in the size of 1-2 cm (Table 1).

Table 1 Ingredient and chemical composition of the experimental diets

Feeds	Percentage of dry matter
Alfalfa hay	30.0
Corn grain	20.0
Barley grain	24.0
Wheat bran	14.0
Soybean meal	9.7
Vitamins and minerals supplement[1]	1.0
Salt	0.5
Limestone	0.5
Dicalsiumphosphate	0.3
Chemical composition (basis dry matter)	
Dry matter (%)	90.00
Crude protein (%)	18.32
Ether extract (%)	3.54
Neutral-detergent fiber (%)	20.74
Acid-detergent fiber (%)	9.80
Ash (%)	7.30
Metabolizable energy (Mcal/kg DM)	2.10

[1] Vitamin-trace mineral pre-mix provides per kg of mixed ration: vitamin A: 1000000 IU; vitamin D3: 75000 IU; Antioxidants: 3000 (mg); Ca: 150000 (mg); P: 60000 (mg); Mg: 300009 (mg); Mn: 2000 (mg); Fe: 3000 (mg); Cu: 500 (mg); Zn: 2500 (mg); Co: 10 (mg) and I: 20 (mg).

Feed intake, performance and digestibility

Due to need for calculate feed intake and FCR (kg DMI/kg live mass gain) during the 45-day trial, feed intake and leftover were recorded daily and DM Intake was calculated by determination of the ration dry matter (60 ˚C, 48 h). To evaluate the growth performance including live weight (LW) changes and feed conversion ratio (FCR). The animals were weighed at the beginning of the experiment (with the starvation 8 h before the start of the experiments) and then every week. The BW changes were calculated by difference of final and initial weight. A digestion trial for 7 days (sample collection) was conducted at the end of experimental feeding, during which daily feed intake and feces excretion were collected and recorded. Samples (about 10%) of feed, leftover and feces were collected every morning and stored in a freezer at -20 ˚C. Feces were collected using a total collection method over 24 h. The feed, feces and orts samples for 7-day collection were pooled, oven dried (60 ˚C, 48 h), ground to pass through a 1-mm screen and stored for chemical analysis. All samples were analyzed for AOAC (2005).

Blood samples collection and hormone

Blood samples from jugular vein were collected in serum tubes (Damoon-E70, Iran) contain anticoagulant agent approximately 4 h after morning feeding (Coverdale *et al.* 2004) from all animals at the final day of the experiment. Collected samples were centrifuged for 3000×g at 4 ˚C for 15 min (Hermel, Germany) and separated plasma stored in -20 ˚C until further analysis for, glucose, blood urea nitrogen, triglycerides, total protein, albumin, Globulin and cholesterol were measured using quantity detection kit (Pars azmun company, Iran) and spectrophotometer (model S Bio-Rad Libra. England), respectively. Enzyme activities of glutathione peroxidase, B-hydroxybutyrate, malonedialdehyde and non-esterified fatty acids were also measured using a kit (Rancel, product of Randox Company) according to the manufacturer's instructions and by a spectrophotometer. The concentration of tetraiodothyronine and triiodothyronine hormones was measured using the kit of Antigen Gostar Company by the Elizarider device.

Measurement of skeletal growth index

For body length, height, abdominal circumference, chest circumference, distance between two horns, distance between two eyes and muzzle width at the end of the day was done by standard meter and oblique (Khan *et al.* 2007).

Fecal score measurement

Calves' feces were randomly evaluated three days a week. Stool grades were determined based on 1- hard and consistent, 2- soft and loose, 3- loose and watery, 4- watery with some blood and 5- watery with blood and mucus (Khan *et al.* 2011).

Statistical analysis

Data were analyzed as a completely randomized design to 4 treatments and 7 replicates of treatments using GLM procedure of SAS (2005). A comparison of means by Duncan's multiple range tests was carried out at the probability of 5% level.

RESULTS AND DISCUSSION

The results related to the investigation of vitamin E and selenium supplemental injection in transition period cows and its effects on the performance of newborn calves are shown in Table 2. As can be seen, the injection of 30 and 45 ml of vitamin E and selenium supplements in pregnant cows could significantly increase the final weight and increase the daily weight of newborn calves compared to other groups ($P<0.05$). While the birth weight, dry matter consumption and food conversion ratio were not significantly affected by vitamin E and selenium supplementation during the drought period ($P>0.05$). The results related to the digestibility of dry matter showed that the injection of vitamin E and selenium supplements during pregnancy could not affect the digestibility ($P>0.05$).

The results related to the injection of vitamin E and selenium supplements in pregnant cows and its effects on the blood parameters of newborn calves are shown in Table 3. As can be seen, the blood concentration of glucose, cholesterol, blood urea, total protein, albumin and the ratio of albumin to globulin of newborn calves could not be significantly affected by vitamin E and selenium supplementation. However, the blood concentration of triglycerides of newborn calves with injection of 15, 30 and 45 ml in dry season cows showed a significant increase compared to the control group (P<0.05).

The results related to the injection of vitamin E and selenium supplements in pregnant cows and its effects on the blood concentration of enzymes and triiodothyronine and tetraiodothyronine hormones in newborn calves are shown in Table 4. The results showed that the injection of vitamin E and selenium in pregnant cows increased the concentration of gutathione peroxidase in calves after birth (P<0.05). Also, the blood concentration of hormones showed that the injection of 15 mL of vitamin E and selenium supplements increases the concentration of triiodothyronine and tetraiodothyronine in infant calves (P<0.05). While the content of beta-hydroxybutyrate, malondialdehyde and non-esterified fatty acids of the calf could not be affected by vitamin E and selenium supplementation (P>0.05).

The results related to the investigation of vitamin E and selenium supplementation in cows during the transition period and its effects on the skeletal growth of the calves born from them are shown in Table 5. As can be seen, the injection of vitamin E and selenium supplements in pregnant cows could not have a significant effect on the body length, height at the withers, belly circumference and chest circumference of the calves born from them (P>0.05).

The results related to the investigation of vitamin E and selenium supplementation in cows during the transition period and its effects on the condition of diarrhea and stool score of the calves born from them are shown in Table 6. The fecal score of newborn calves could not be affected by vitamin E and selenium supplementation. Also, there was no significant difference in the number of diarrhea attacks and the average days of diarrhea in the calves born (P>0.05).

Consistent with the present results, Moeini *et al*. (2009) and Cohen *et al*. (1991) reported that the injection of 20 and 40 ml of vitamin E and selenium solution at 4 and 2 weeks before calving did not affect the birth weight of calves. Also, in another study, Daugherty *et al*. (2002) did not observe any effect on the birth weight of calves by injecting mineral solutions in pregnant cows that had antioxidant properties. Mousavi *et al*. (2019) showed that the injection of vitamin E and selenium in the rations of cows during the transition period did not affect the birth weight of calves.

Soliman *et al*. (2012) injected vitamin E and selenium solution into pregnant ewes, the growth performance of lambs born from them improved. In a research, Shi *et al*. (2011) observed a positive effect on growth performance by feeding selenium in goats. These researchers explained the increase in dietary selenium and its absorption as the reason for this growth. Also, in another study, Al-Shahat and Abdul Monem (2011) reported that supplementing the diet with vitamin E and selenium in the diet of ewes, the growth performance of the born lambs increased. Kafilzadeh *et al*. (2014) did not observe any effect on birth weight, daily weight gain and weaning weight in calves born with vitamin E and selenium injection and oral methods. Similarly, in another study, Droke and Loerch (1989) in calves, Mohri *et al*. (2011) in Balochi lambs, Voudouri *et al*. (2003) did not observe a positive effect on growth performance using vitamin E and selenium. In the present study, the cows that were injected with 30 mL of vitamin E and selenium had a numerically higher birth weight, and the final weight was also higher in this treatment, considering that selenium can be transferred to the fetus through the placenta and colostrum to the calf. It is possible that the injection of vitamin E and selenium has increased the absorption of selenium from the placenta to the fetus, and as a result, the final weight of the calves has improved. On a cellular level, dietary Se may influence various leukocytic effector functions including adherence, migration, phagocytosis, and cytokine secretion. Several members of the selenoprotein family regulate or are regulated by cellular redox tone, which is a crucial modulator of immune cell signaling and function. Supplementation of vitamin E significantly enhances both cell mediated and humoral immune functions in humans, especially in the elderly and animals (Droke and Loerch, 1989).

As can be seen, the blood concentration of glucose, cholesterol, blood urea, total protein, albumin and the ratio of albumin to globulin of newborn calves could not be significantly affected by vitamin E and selenium supplementation. However, blood triglyceride concentration of newborn calves with 15, 30 and 45 mL injections in pregnant cows showed a significant increase compared to the control group. In line with the present results, Tanaka *et al*. (2001) reported that the use of vitamin E and selenium supplements in dairy cows did not have a significant effect on the concentration of blood albumin, total protein and globulin. Also, these researchers stated that the use of vitamin E and selenium in pregnant cows, it could not have an effect on the blood concentration of glucose and cholesterol in the calves born from them. In a research, Juniper *et al*. (2006) also stated the lack of effect of selenium consumption on blood albumin concentration in dairy cows.

Table 2 Injection of different levels of vitamin E and selenium supplementation in pregnant cows and its effects on the performance of newborn calves

Functional characteristics	Control	15 mL	30 mL	45 mL	SEM	P-value
Initial weight (kg)	39.98	40.28	41.28	40.88	0.621	0.720
Final weight (kg)	59.55^{b}	60.72^{ab}	63.56^{a}	63.42^{a}	1.062	0.032
Total weight gain (kg)	19.57	20.44	22.28	22.54	0.611	0.097
Daily weight gain (g)	434.48^{b}	454.22^{b}	495.11^{a}	501.11^{a}	12.720	0.039
Dry matter intake (g)	139.50	141.25	149.66	150.13	5.897	0.387
Total dry matter intake (kg)	6.27	6.35	6.73	6.75	0.759	0.111
Feed conversion ratio	3.11	3.21	3.30	3.33	0.471	0.132
Dry matter digestibility (g/kg)	737.75	745.16	734.25	780.50	23.712	0.067

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Injection of different levels of vitamin E and selenium supplementation in pregnant cows and its effects on the blood parameters of newborn calves

Blood parameters	Control	15 mL	30 mL	45 mL	SEM	P-value
Cholesterol (mg/dL)	75.01	75.37	78.55	77.93	2.895	0.745
Triglyceride (mg/dL)	37.91^{C}	43.93^{a}	40.94^{b}	40.94^{b}	0.995	0.004
Glucose (mg/dL)	83.75	83.11	88.26	86.47	2.824	0.488
Urea (mg/dL)	10.12	10.78	10.91	10.00	0.598	0.614
Total protein (g/dL)	7.24	7.78	7.25	7.64	0.660	0.502
Albumin (g/dL)	4.55	4.88	4.34	4.60	0.391	0.517
Globulin (g/dL)	2.69	2.90	2.91	3.04	0.474	0.630
Albumin:globulin	1.69	1.68	1.49	1.51	0.401	0.207

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Injection of different levels of vitamin E and selenium supplementation in pregnant cows and its effects on the hormonal parameters of newborn calves

Hormonal parameters	Control	15 mL	30 mL	45 mL	SEM	P-value
Glutathione peroxidase activity (U/gHb)	297.82^{b}	451.21^{a}	409.28^{a}	419.87^{a}	16.722	0.001
Triiodothyronine (ng/dL)	157.14^{c}	248.78^{a}	218.19^{b}	212.21^{b}	10.741	0.002
Tetraiodothyronine (µg/dL)	4.87^{b}	5.42^{a}	5.61^{a}	5.52^{a}	0.067	0.004
B-Hydroxybutyrate (mmol/L)	0.20	0.19	0.16	0.18	0.009	0.874
Malone di aldehyde (ng/g)	1.12	1.06	1.06	1.18	0.041	0.117
Non-esterified fatty acids (mmol/L)	0.21	0.17	0.20	0.16	0.029	0.674

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Injection of different levels of vitamin E and selenium supplementation in pregnant cows and its effects on the skeletal indicators of newborn calves

Skeletal indicators	Control	15 mL	30 mL	45 mL	SEM	P-value
Body length (cm)	109.71	109.51	109.51	109.31	0.962	0.962
Front height (cm)	94.11	94.11	95.10	94.31	0.681	0.772
Belly girtht (cm)	106.71	106.01	106	106.21	0.775	0.574
Chest girth (cm)	102.91	104.11	104.10	104.31	0.794	0.790
Horns distance (cm)	12.04	12.24	12.23	12.44	0.194	0.461
Ayes distance (cm)	14.51	14.71	14.70	14.91	0.170	0.810
Muzzle width (cm)	6.94	7.14	7.19	7.24	0.102	0.474

SEM: standard error of the means.

Table 6 Injection of different levels of vitamin E and selenium supplementation in pregnant cows and its effects on the diarrhea condition of newborn calves

Diarrhea condition	Control	15 mL	30 mL	45 mL	SEM	P-value
Score faeces	1.48	1.40	1.44	1.42	0.061	0.642
Number of calves with diarrhea	4	3	3	4	0.251	0.412
Average days of diarrhea	1.50	1.75	1.50	1.50	0.207	0.584

SEM: standard error of the means.

Also, Haidari *et al.* (2012) showed that the blood concentration of triglycerides and cholesterol could not be significantly affected by intramuscular injection of 1 cc of vitamin E and selenium in Baluchi sheep. The reports of Zarei *et al.* (2020) by examining the effects of selenium supplementation in the diet of suckling calves showed that selenium supplementation increases the concentration of glucose. In another study, Asadi *et al.* (2018) showed that the injection of vitamin E and selenium in lambs increased blood glucose and triglyceride levels. Slavik *et al.* (2008) and Shinde *et al.* (2009) showed that the use of selenium has no effect on blood protein concentration. Also, Singh *et al.* (2002) and Kumar *et al.* (2009) reported that supplementing the diet with selenium did not affect globulin concentration. Hisham *et al.* (2016) reported that the use of vitamin E and selenium supplements did not show an effect on blood glucose concentration. Calamari *et al.* (2011) found that the use of vitamin E and selenium injections or the use of sodium selenite or vitamin E does not have a significant effect on the blood concentration of glucose, cholesterol and triglycerides. Contrary to the results of this research, Falkowska and Minakowski (2000) and Antunović *et al.* (2014) stated that supplementing the diet with selenium does not have a significant effect on the blood concentration of triglycerides.

Glutathione peroxidase is an antioxidant enzyme containing selenium, and there is a direct relationship between the increase in the activity of this enzyme and the concentration of vitamin E and serum selenium. Glutathione peroxidase converts free radicals into weaker metabolites and thus protects the tissue against oxidative damage (McKenzie *et al.* 2002). In a study, Aliarabi and Fadayfar (2016) showed that the use of selenium and vitamin E supplements in the form of injections or slow-release tablets in pregnant ewes and lambs born from them increases the blood concentration of glutathione peroxidase. Also, in another study, Lacetera *et al.* (1996) by injecting a solution of vitamin E and selenium (25 international units and 5 mg of selenium per 100 kg of body, respectively) in dairy cows on days 22 and 11 before calving showed that the activity of glutathione peroxidase enzyme in the blood of calves has increased in 48 hours after birth. On the other hand, Mousavi *et al.* (2019) stated that the injection of vitamin E and selenium solution in pregnant cows had no effect on the concentration of glutathione peroxidase enzyme in calves. In another study, Melanie *et al.* (2017) reported that the injection of vitamin E and selenium in transition period cows increased the blood glutathione peroxidase concentration on the day of calving. Mohrekesh *et al.* (2018) two intramuscular injections of vitamin E and selenium (2100 mg of vitamin E and 7 g of selenium sodium selenite) in two weeks before calving and the day of calving in dairy cows on plasma vitamin E concentration and activity Blood glutathione peroxidase enzyme had no effect in the 2nd and 8th week after birth. In the study of Balicka-Ramisz and Jastrzębski (2014), the effect of supplementing mineral elements including selenium on days 230 and 260 of pregnancy on serum glutathione peroxidase enzyme activity on day 6 before delivery and days 3, 7, 14 and 25 after delivery has a significant effect. It was not surprising that these researchers considered the reason for it to be the long interval between the time of injection and sampling. Abdolmaleki *et al.* (2017) reported that the injection of vitamin E and selenium in cows during the transition period could not have a significant effect on the concentration of malondialdehyde in cows and calves born from them. Asadi *et al.* (2018) reported that the use of selenium and vitamin E supplements by injection or orally in infant lambs increases the conversion of the hormone tetraiodothyronine to triiodothyronine. Also, in another study, Soliman *et al.* (2012) reported that injection of one milliliter of vitamin E and selenium solution in pregnant ewes increases the amount of triiodothyronine hormone. Selenium plays a role in the normal metabolism of thyroid hormone, so the existence of this element is necessary for the conversion of tetraiodothyronine hormone into triiodothyronine through the type 4 deiodinase enzyme (Asadi *et al.* 2021b). Selenoperoxidases protect the thyroid gland against the peroxides produced during the synthesis of hormones, which shows the importance of this element in regulating the state of the thyroid in animal tissues (Lesmeister and Heinrichs, 2004).

The injection of vitamin E and selenium supplements in pregnant cows could not have a significant effect on the body length, height at the withers, abdominal circumference and chest circumference of the calves born from them. Ziaei (2015) reported that the use of different sources of selenium affected the body length and breast circumference of weanling calves. In agreement with the present study, Gunter *et al.* (2003) and Rowntree *et al.* (2004) showed that supplementing the diet with selenium could not improve growth parameters. Sasani *et al.* (2015) reported that the injection of vitamin E and selenium in infant calves did not cause significant differences in skeletal growth indices (chest circumference, withers height, hip height, body length and distance between two hip bones). Asadi *et al.* (2021b) showed that the intake of selenium and vitamin E by injection and orally and according to the recommendations of NRC and ARC compared with the control group in terms of length, frontal height, abdominal circumference, chest circumference, distance between two horns, distance between Eyes and muzzle width do not make a significant difference in suckling calves.

These researchers stated that changes in skeletal growth are affected by the state of growth and health of the herd, genetics, feed consumption and feed conversion ratio.

The fecal score of newborn calves could not be affected by vitamin E and selenium supplementation. Also, there was no significant difference in the number of diarrhea attacks and the average days of diarrhea in the calves born. Diarrhea is one of the most important diseases in suckling calves, which causes the death of calves and affects the health and economy of the herd (Ghosh *et al.* 2011). In the early ages when the consumption of starter feed is very low, the stool and diarrhea score of calves is affected by physiological, environmental, health and management factors (Ghosh *et al.* 2010) and is less affected by the type and composition of starter feed (Lesmeister and Heinrichs, 2004). In line with the results of the present experiment, Zarei *et al.* (2020) reported that the addition of selenium supplement in the diet of infant calves could not improve the health status and stool score. Also, Asadi *et al.* (2021b) showed that the intake of selenium and vitamin E by injection and orally, according to the recommendations of NRC and ARC, does not create a significant difference with the control group in terms of stool score, the number of calves suffering from diarrhea, and the days of calves suffering from diarrhea. According to the results of the present experiment, the consumption of selenium and vitamin E did not affect the state of diarrhea and stool consistency, while Ishikawa (1993) observed in a study on infant calves that the levels of selenium and vitamin E were low in calves with persistent diarrhea. He reported that there were no detectable specific pathogens in these calves. All the calves with diarrhea responded positively to the treatment in which selenium and tocopherol were used, so in cases of continuous diarrhea in calves, the deficiency of these two antioxidant substances can also be considered. Mohrekesh *et al.* (2018) reported that the injection of selenium supplements in pre-partum cows can have a positive effect on the health status of the calves born. Also, Sasani *et al.* (2015) stated that the use of selenium and vitamin E injection supplements improved the stool fluidity coefficient among the calves receiving this supplement.

CONCLUSION

The results of this experiment showed that the injection of vitamin E and selenium in pregnant cows could not improve blood parameters including glucose, cholesterol, urea, total protein, albumin, globulin and growth performance including dry matter consumption, birth weight, feed conversion ratio, digestibility. Dry matter affects skeletal growth and health status. While the injection of vitamin E and selenium solution in the prenatal period could improve daily weight gain, blood concentration of glutathione peroxidase and triiodothyronine and tetraiodothyronine hormones. According to the present results, it is suggested to inject at least 30 ml of selenium and vitamin E supplements before calving in pregnant cows.

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