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The Effect of Enriched Milk with Selenium and Vitamin E on Growth Rate, Hematology, Some Blood Biochemical Factors, and Immunoglobulins of Newborn Goat Kids

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Abstract Thirty male and female (n=15 for each one) Markhoz newborn goat kids (aged 7±3 days) were distributed in a randomized block design in a 2×2+1 factorial arrangement: two levels of sodium selenite as a source of selenium (0.2 or 0.3 ppm Se), two levels of α-tocopherol acetate as a source of vitamin E (150 or 200 IU Vit E), and one control treatment with six repetitions per treatment (each replicate included three male and three female kids). Animals were fed daily by Se-Vit E-enriched milk (Se-Vit E treatments) or non-enriched milk (control treatment). Growth rate, hematology, and serum biological parameters were measured. The levels of serum albumin (P<0.01), serum globulin (P<0.05), total serum protein levels (P<0.01), erythrocyte counts (RBC) (P<0.001), hemoglobin (P<0.001), hematocrit (P<0.001), leukocyte counts (WBC) (P<0.001), IgA (P<0.05), IgG (P<0.01), and IgM (P<0.01) significantly differed among treatments, while no significant differences were observed for calcium, lymphocyte, neutrophil average daily gain and body weight among treatments. Kids feeding by enriched milk with 0.3 ppm Se and 200 IU Vit E had significantly higher serum total protein, globulin, RBC, IgA, IgG, and IgM compared to control and those fed by enriched milk to 0.2 ppm Se and 200 IU Vit E had significantly higher WBC counts.

Keywords Selenium · Vitamin E · Growth rate · Hematology · Immunoglobulins · Newborn goat kids

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Introduction

Goat kids are exposed to a variety of physiological and pathological stresses which caused high mortality rates in the first weeks of their lives [1]. Nutritional strategies are necessary to reduce the immunosuppressive consequences of physical or pathological stressors. Preweaning supplementation of selenium (Se) and vitamin E (Vit E) completely diminish the immune debilitation commonly concerned with the physiological consequences of stress; moreover, because of Se deficiency in soils of many regions of Iran and insufficient dietary Se from feedstuffs which grown on these soils [2], newborn goat kids could not receive sufficient amount of Se from their mothers. This could negatively affect resistance in kids and cause health problems and high mortality rates. Vit E does not cross the placenta in substantial quantities; then, it makes newborns highly susceptible to Vit E deficiency [3].

Se and Vit E have a close relationship with each other. Both Se and Vit E exert similar antioxidant effects in cells, but via independent biochemical pathways and in different locations [3]. Se is involved in the protection of biological membranes against oxidation by hydrogen peroxide and other oxidizing agents, e.g., free radicals, superoxide, and organic hydroperoxide [4] because it is a substantial constituent of glutathione peroxidase which destruct these components in the cytoplasm and protect tissues against oxidative damage. Se is required for growth, fertility, and prevention of various diseases including nutritional muscular dystrophy or white muscle disease [4, 5]. It has an essential role in generation of resistance to disease either through improving the immune response, leukocyte function, or specialized immunity of the animals [4]. It is approved that inadequate dietary consumption of Se is also related to several disorders of ruminants such as ill-thrift

syndrome, neonatal weakness, calf pneumonia, sudden death, and immune suppression [6].

Vit E has been recognized as an essential nutrient for growth and immunity of all species of animals [3]; the diverse roles of Vit E are due to its involvement in nutritional myopathy, prostaglandin biosynthesis, and immune responsiveness [7, 8]. An insufficient supply of Vit E, especially in newborns, is a frequent cause of immunodeficiency [9] and also could affect on humoral immunity [10] and on cell-mediated immunity, which are responsible for protection against viral, protozoal, and fungal infections.

This research was conducted on newborn goat kids to find out the effects of enriched milk with different levels of Se and Vit E on their growth rate, some blood biochemical factors, hematology, and immunoglobulins changes.

Materials and Methods

Animals and Grouping

This experiment was performed in Markhoz goat research station of Sanandaj at Kurdistan Province in the west of Iran. A total 30 male (n=15) and female (n=15) newborn Markhoz goat kids (about 7±3 days of age) were randomly allocated to five treatments as the numbers of males and females were equal in each treatment (Table 1). The kids in each treatment were kept with their mothers in separate pens until the end of the experiment; before feeding enriched milk, goat kids were kept separate from their mothers for 3 h and fasted, and then they were fed by Se-Vit E-enriched milk by pacifiers. Vit E as a form of α-tocopherol acetate and Se as a form of sodium selenite were fully mixed with 70 cc milk expressed from each mother. Required milk was milked from mothers and labeled based on kid and its mother tag number and transferred to the laboratory. To avoid Se toxicity to kids, Se based on the dose related to each treatment was solved completely with 10 cc of milk with an electric mixer fitted with a heater (heating was at 37 °C near to body temperature) then the solution was mixed with regarding Vit E dose plus 60 cc of milk with an electric mixer.

Weighing and Sampling

Animals were weighted weekly from the beginning to the end of the experiment (8 weeks). About 10 ml of blood samples was collected from each kid via the jugular vein at the end of the study. Two and half milliliters of blood anticoagulated with EDTA was used for counting of blood cells and 7.5 ml of sample was transferred to a plane tube for serum separation. All tubes were instantly kept at 4 °C; plane tubes were centrifuged (3,000×g for 10 min) and the following serum was separated, and then, all samples were transferred to the laboratory and stored at −20 °C until analyses.

Table 1 Schematic presentation of treatments administered

	Control	T1	T2	T3	T4
Selenium (ppm)	0	0.3	0.2	0.2	0.3
Vitamin E (IU)	0	200	150	200	150

Anticoagulated blood was analyzed after collection for measurements of hematocrit (PCV), hemoglobin (Hb), leukocyte and erythrocyte counts (WBC and RBC) by microhematocrit, cyanmethaemoglobin, and standard manual methods, respectively. Serum total protein was detected by biuret colorimetric method using a spectrophotometer. Albumin and calcium were measured by commercial kits (Farasmad, Tehran, Iran). The concentration of globulin was calculated as the difference between serum total protein and albumin. The serum immunoglobulin concentrations (IgA, IgG, and IgM) were assessed by the Sandwich ELISA method using the commercial Kits (Pars Azmun K2055008-10)®. Average daily gain (ADG) and total gain ratios were calculated for all days of the research period.

Statistical Analysis

Data analysis was performed by general linear model procedure of SAS (SAS institute, 2003) as a randomized complete block experiment. The model consisted of treatment (Table 1), sex of kids, treatment and sex interaction, random effect of animals within treatment, and residual error, with individual animals considered the experimental units.

Once again, all data were subjected to completely randomized block design with a 2×2 factorial analysis; in this analysis, control treatment was not taking into account, then analysis was performed using data of other treatments. The model contained the effects for Se, Vit E, sex of kids, and all interactions. Means were separated by LSD, and least squares means and SEM for all data are presented. The main effects were discussed if P<0.05.

Results

Weight Gain

The initial body weight of the animals showed no significant differences among treatments (Table 2) and different levels of Se and Vit E levels (Table 3). The weight gain was not significantly different among treatment groups, although weight gain in Se and Vit E-treated animals was higher than those in control group (Table 2). The rate of weight gain in females was higher than males (P=0.09) and a significant interaction between sex and treatment was

Table 2 Mean of initial body weight, total gain, and ADG parameters in different treatments and sexes

	Control	T1	T2	T3	T4	SEM	Male	Female
Initial body weight (kg)	4.66	4.79	4.79	5.13	5.05	0.107	4.79±0.138	4.98±0.165
Total gain (kg)	2.30	2.95	2.52	3.058	2.84	0.127	2.56±0.106	2.91±0.226
ADG (g/day)	54.89	70.35	60.04	72.82	67.65	3.028	61.00±2.532	69.30±5.395

observed ($P<0.05$) (Table 2). The live weight gain and ADG were not statistically significant between animals receiving different levels of Se and Vit E levels, although animals given 0.3 or 200 IU Vit E had higher ADG and total gain (Table 3).

Calcium and Serum Proteins

Serum concentrations of calcium were not significantly different among treatments, although were greater in the treated kids than the control group (Tables 4 and 5). The serum levels of albumin ($P<0.01$), globulins ($P<0.05$), and total protein ($P<0.01$) were significantly different among treatments. Albumin was significantly decreased in treatments 2 and 3 compared to the control. Globulin was higher in treatments 2 and 4 than the control; serum total protein significantly increased in Se and Vit E-supplemented groups (Table 4). Serum albumin in animals receiving 0.3 Se was significantly higher than animals receiving 0.2 Se. Kids supplemented with 150 IU Vit E had significantly higher serum total protein than those supplemented with 200 IU Vit E. There were not any significant differences in serum globulin between kids supplemented with different levels of Se or Vit E (Table 5). Both males and females had not any significant differences relating to above parameters (Table 4).

Hematology

Blood parameters such as RBC, Hb, PCV, and WBC concentrations were different among treatment groups ($P<0.001$), while no significant differences were observed for lymphocytes and neutrophils counts. RBC, Hb, WBC, and PCV were highest in treatments 1, 4, 3, and 1, respectively (Table 4). Supplementation of 200 IU Vit E significantly increased Hb ($P<0.01$), PCV ($P<0.05$), and WBC ($P<0.001$) compared to supplementation of 150 IU Vit E. RBC, Hb, PCV, lymphocyte, and neutrophil ($P>0.05$) were not significantly different among Se-supplemented kids, but WBC was significantly higher in kids given 0.2 ppm Se than those given 0.3 ppm Se ($P<0.01$) (Table 5). There were no significant differences between sexes in all mentioned parameters.

Immunoglobulins

Significant differences were seen for IgA ($P<0.05$), IgG ($P<0.01$), and IgM ($P<0.01$) among treatments. IgA, IgG, and IgM were highest in treatment 2 (Table 6). Immunoglobulin levels were not significantly differed between kids given with different levels of Se or Vit E ($P>0.05$) (Table 7). We had not observed any mortality among treatment groups.

Discussion

Controversial results exist concerning the effect of Se supplement on weight gain in different animals. Similar to the results of the current study, no effect of Se supplement on growth rate and weight gain in cattle [11–13], sheep [14], pig [15–17], buffalo [18], and chicken [19] was found. In contrary with our results, positive responses to the Se supplement on ADG have been reported in sheep [20–23], goat [24], cattle [25, 26], and chicks [27]. Analogously, supplemental selenomethionine (0.3 to 0.5 mg Se/kg DM) significantly increased ADG and feed efficiency compared with the control group in Taihang Black goats [28]. Similarly, inorganic, organic, and elemental nano-Se (0.3 mg/kg Se) diet supplementation to growing male goats increased final body weight and ADG; however, the enhancing effect of organic and elemental nano-Se on ADG was greater [29]. In agreement with our data, there were no significant differences in growth rate between 0.15 and 0.30 ppm Se-

Table 3 Mean of body weight, total gain, and ADG parameters in newborn goat kids fed by enriched milk with different levels of Se and Vit E

	Se		Vitamin E	
	0.2	0.3	150	200
Initial body weight (kg)	4.96±0.157	4.92±0.176	4.92±0.185	4.96±0.147
Total gain (kg)	2.79±0.167	2.89±0.235	2.68±0.212	3.00±0.183
ADG (g/day)	66.43±3.98	69.01±5.59	63.85±5.05	71.59±4.37

Table 4 Mean concentration of different blood biochemical and hematology factors in different treatments and sexes

	Control	T1	T2	T3	T4	Male	Female
Albumin (g/dl)	3.65±0.034a	3.53±0.021ab	3.46±0.042b	3.43±0.066b	3.65±0.076a	3.53±0.038	3.56±0.04
Globulin (g/dl)	2.40±0.063B	2.75±0.123AB	3.18±0.101A	2.88±0.113AB	2.90±0.124A	2.86±0.092	2.78±0.094
Serum total protein (g/dl)	5.71±0.153b	6.23±0.088a	6.46±0.084a	6.26±0.136a	6.48±0.087a	6.28±0.11	6.18±0.08
Calcium (mg/dl)	8.89±0.204	9.18±0.153	9.24±0.109	9.16±0.096	8.96±0.155	9.16±0.090	9.01±0.97
RBC ($\times 10^6$/μl)	15.42±0.173b	17.51±0.392a	15.53±0.451b	15.41±0.661b	14.03±0.360b	15.68±0.404	15.48±0.383
Hb (g/dl)	9.01±0.087a	9.70±0.129a	9.23±0.178a	9.26±0.234a	7.91±0.279b	8.51±0.198	8.69±0.194
PCV (%)	27.71±0.905ab	30.23±1.002a	29.33±0.465a	29.63±0.438a	25.63±0.760b	27.98±0.644	29.04±0.584
WBC ($\times 10^6$/μl)	18.53±1.657bc	22.5±1.654ab	19.41±1.849bc	26.45±0.645a	16.21±0.906c	21.27±1.256	19.97±1.265
Lymphocyte (%)	32.16±1.44	35.33±1.229	35.83±1.30	36.33±1.706	31.83±1.661	35.2±0.857	33.4±1.111
Neutrophils (%)	65.5±1.384	63.83±1.492	66.66±0.557	63±1.6329	66.33±1.686	64.33±0.908	65.8±0.900

Means in same row with different lowercase letter are significantly different ($P<0.01$). Means in same row with different capital letters are significantly different ($P<0.05$)

supplemented lambs [22]. In accordance with result of present study, Smolders et al. [30] reported that parenteral administration of 0.5 ml Vit E and Se solution (treated group) or 0.5 ml salt solution (placebo) combined with powdered full goat milk or goat milk replacer during the raising period at the day of birth had not any significant effect on ADG.

Numerous studies have addressed the effects of Se and Vit E on blood proteins. In research studies in steers [31], buffaloes [32], lambs [22], and rats [33], no differences in serum total protein, albumin, globulin, and albumin/globulin ratio was distinguished due to Se supplementation. Consistent with our results, combined injection of Vit E plus Se to ewes led to significant increases in plasma total protein and globulin for ewes and their lambs. Similar to results of current study, changes in plasma albumin concentration for treated ewes and their lambs were not significant [34]; similar results were noted in other research which reported that Baladi ewes supplemented with 50 mg Vit E plus 0.3 mg of Se/kg diet, at 2 weeks before mating and extended through pregnancy till lambing, resulted in a significant increase in total serum protein and globulin [35]. In an experiment, supplementation of Se to male buffalo calves had no significant effects on concentrations of total protein, but similar to our observations, the level of globulin was significantly increased, leading to reduced levels of albumin [36]. In another study, these researchers found that supplementation of Se, Cu, and Zn had not any significant effect on albumin, globulin, total protein, and other investigated blood biochemical parameters in the same animals [37]. The information pertaining to the effect of Se and Vit E supplementation on calcium is limited. The presented results are in agreeable with the finding of Kumar et al. [22], who reported that Se supplementation had not any effect on calcium intake in lambs, although, similar with our data, the calcium levels were higher in the treated groups than in the control. In another research, intramuscular injection of Se and Vit E to calves had no significant effect on serum contents of calcium, magnesium, sodium, potassium, copper, iron, and zinc [38]. Surprisingly, Mahmoud et al. [39] recently found that calcium levels significantly increased in

Table 5 Mean concentration of different blood biochemical and hematology factors in newborn goat kids fed by enriched milk with different levels of Se and Vit E

	Se		Vitamin E	
	0.2	0.3	150	200
Albumin (g/dl)	3.45±0.0379b	3.59±0.0417a	3.55±0.0499	3.48±0.0366
Globulin (g/dl)	3.03±0.0856	2.82±0.0863	3.04±0.0874	2.81±0.0824
Serum total protein (g/dl)	6.37±0.082	6.36±0.07	6.47±0.0579a	6.25±0.0774b
Calcium (mg/dl)	9.23±0.1171	9.08±0.0912	9.13±0.1181	9.18±0.0946
RBC ($\times 10^6$/μl)	15.142±0.636	14.60±0.928	14.28±0.668	15.46±0.877
Hb (g/dl)	9.25±0.140	8.80±0.306	8.57±0.253b	9.48±0.143a
PCV (%)	29.48±0.308	27.93±0.916	27.48±0.701B	29.93±0.529A
WBC ($\times 10^6$/μl)	22.93±1.41a	19.35±1.306b	17.81±1.094b	24.47±1.035a
Lymphocyte (%)	36.08±1.025	33.58±1.117	33.83±1.173	38.06±1.013
Neutrophils (%)	64.83±0.991	65.08±1.137	66.5±0.848	63.41±1.062

Means in same row with different lowercase letter are significantly different ($P<0.01$). Means in same row with different capital letters are significantly different ($P<0.05$)

Table 6 Mean concentration of immunoglobulins in different treatments and sexes

	Control	T1	T2	T3	T4	Male	Female
IgA (g/dl)	0.073±0.003B	0.083±0.004AB	0.096±0.003A	0.088±0.004AB	0.086±0.004AB	0.087±0.002	0.084±0.003
IgG (g/dl)	1.92±0.050b	2.20±0.098ab	2.54±0.081a	2.30±0.091ab	2.32±0.099ab	2.29±0.073	2.22±0.075
IgM (g/dl)	0.411±0.011b	0.468±0.020ab	0.54±0.0171a	0.49±0.0179ab	0.49±0.021ab	0.49±0.015	0.44±0.015

Means in same row with different lowercase letters are significantly different ($P<0.01$). Means in same row with different capital letters are significantly different ($P<0.05$)

rams supplemented with 5 mg sodium selenite and 450 mg Vit E for 1 month.

There are controversial results in regard to the effects of Se and Vit E supplementation on hematology. In contrary to the results of this study, injection of Se and Vit E had not any significant effect on RBC count, Hb concentration, and PCV value in rats [33] and calves [38]. Furthermore, Mohri et al. [40] reported that Se and Vit E injection (α-tocopherol acetate 3.82 g/100 ml plus sodium selenite 0.023 g/100 ml) had not any significant effect on WBC, neutrophil, lymphocyte, and monocyte counts between control and test groups of lambs, but in disagreement with our data that Hb was decreased in the treated group compared with the control lambs. None of the hematological factors (RBC, Hb, PCV count) were affected by supplementation of Vit E and Se in male broiler chicken but ascorbic acid, aspirin, Vit E, and Se supplementation significantly decreased the WBC counts [41]. Moeini et al. [42] found that prepartum supplementation of Se and Vit E affected WBC and neutrophil counts in calves. In another study, Faixova et al. [43] showed that Se-yeast supplementation to lambs significantly increased RBC and decreased WBC counts. In agreement with result of this study, different research studies indicated higher WBC counts in Se-supplemented lambs [44], calves [38, 45], and rats [33]. The results of current study indicated that Se and Vit E affected WBC and RBC cell counts. Enhancing of these cells could be concerned with the conservation of cell membrane and organelles by the antioxidant effects of Se and Vit E and therefore increase their oldness [40]; on the other view, Se and Vit E can affect on hematopoiesis by their antioxidant effects.

Different research studies showed mixed results pertaining to Se and Vit E effect on the content of immunoglobulins: in the current study, the concentration of serum levels of IgA, IgG, and IgM was significantly increased by milk supplementation of Se and Vit E; in accordance, Droke and Loerch [11] and Awadeh et al. [13] indicated that higher levels of Se supplementation led to an increase in serum concentration of IgG in beef cows and their calves. Knight and Tyznik [46] also reported an increase in IgG over time when Se was supplemented to ponies fed a Se-deficient diet. Likewise, supplementary Vit E and Se for cows significantly increased serum contents of IgG and IgM at 12 h postpartum and the colostrum content of IgM, IgG, and IgA in comparison with control cows [26]. On the contrary, no significant differences in concentration of IgG were detected among Se-supplemented and control heifers and their calves [42]. Similarly, supplementation of Vit E to calves had no significant impact on the concentrations of IgG1 and IgG2, but significantly affected on IgM [47]. In another study, Hidiroglou et al. [48] suggested that Vit E-supplemented calves had no significant differences for IgG1, IgG2, and IgM concentrations compared to control group.

A diversity of opinion related to the effects of Se and/or Vit E on investigated parameters in the present study often existed in literature. One of the reasons is the fact that their effects were mostly studied synchronously together or with that of other factors which made the interpretation difficult. In this study, we surveyed the effect of enriched milk with Se and Vit E on mentioned parameters in the newborn goat kids,

Table 7 Mean concentration of immunoglobulins in newborn goat kids fed by enriched milk with different levels of Se and Vit E

	Se		Vitamin E	
	0.2	0.3	150	200
IgA (g/dl)	0.092±0.003	0.085±0.003	0.091±0.003	0.085±0.003
IgG (g/dl)	2.42±0.068	2.26±0.069	2.43±0.070	2.25±0.066
IgM (g/dl)	0.515±0.014	0.481±0.014	0.516±0.014	0.479±0.013

such study was conducted for the first time and its results can be unique.

Conclusion

On the basis of the results present study in Markhoz kids, it may be concluded that different levels of Se and Vit E supplementation had no significant impact on growth rate, calcium, lymphocyte, and neutrophil counts. But it significantly improved serum globulin, total serum protein, RBC, Hb, PCV, WBC, IgA, IgG, and IgM. It could be pointed out that positive effects of Se and Vit E have not been universal among research studies as many factors could affect their outcome such as Se or Vit E status at the time of treatment, duration, and dose of administration. Increase in some of the blood parameters and immunoglobulins could be ascribed to the improvement of immunity by Vit E and Se supplementation which improved the kids' overall health.

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