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INFLUENCE OF SELENIUM WITH VITAMIN E AND COBALT HEAVY PELLETS ON REPRODUCTION AND METABOLIC PROFILES OF EWES

Jan Małecki[1], Eugeniusz Malinowski[2], Kazimierz Supera[2], Aleksandra Balicka-Ramisz[1]

[1]*Department of Animal Hygiene and Prophylaxis, Agricultural University of Szczecin, Poland*
[2]*Experimental Station of Zootechnical Institute, Kołbacz, Poland*

ABSTRACT

The aim of the study was to investigate whether synergism of selenium and cobalt preparations occur if jointly administered to three-year-old ewes of the Suffolk breed. Four groups were formed: Se, Co, Se+Co and control, each of 15 ewes. Blood samples were taken one month before mating and 2 weeks after the second selenium injection (the 3rd month of pregnancy). The results indicate that the fertility, prolificacy and the number of twins in the Se group was the highest (87, 131 and 62 %). However, a higher number of stillborn lambs (18 and 26 %) and lower weaned rate (100 and 92%) were observed in the Se and control groups respectively. Wherefore, the best production results were achieved by the ewes of the Se+Co group (prolificacy 127% and weaned rate 118%). Additionally, the conception rate after the 1st service of the ewes treated with selenium and vitamin E (Evetsel) or cobalt (heavy pellets) preparations was (non-significantly) lower than the results obtained for the Se+Co and control groups (73 and 71 vs. 79 and 76%). In blood serum of the Se+Co group of ewes in the 3rd month of pregnancy the level of albumin, calcium and copper were significantly lower, and alfa and beta globulin were significantly higher compared with the control group. Selenium and cobalt preparations, jointly administered to mother ewes, significantly effected their reproduction and metabolic blood serum profiles.

Key words: ewes, selenium and cobalt, reproduction, weaned rate, blood serum, metabolic profiles

INTRODUCTION

Trace elements deficiency is an important factor in animal husbandry. In North-West Poland, the deficiency of selenium (Se) and cobalt (Co) is a serious problem affecting the effectiveness of animal production [20, 21, 28, 38]. Selenium content of all feeds from North Germany (Schleswig-Holstein) was below recommended values, and most of the other trace elements were marginal [34]. Selenium plays a great biochemical and physiological role in animal health, immunity and nutrition [6, 16, 32], and Se supplements should be included into mineral mixtures.

The U.S. Food and Drug Administration approved Se supplementation to sheep diets from 0.1 to 0.3 ppm and to marginal 0.5 ppm in concentrates [39]. In Poland, 0.15 to 0.25 ppm covered Se requirement of lambs and ewes [26, 39, 40]. It is suggested that priority should be given to development of diagnostic tests to identify these mineral disorders. Combined deficiencies, which are not deductible in experiments using single element supplement, may depress productivity in sheep [22]. Early diagnosis of trace element deficiencies is parnumber important to farm profitability.

Several factors affect the serum Se responses of different animals to specific Se and Co intakes [8]. These factors may include forage types and sources, ruminal environment, supplemental feeding, dietary calcium and trace elements. When lambs were fed on cobalt-deficient green diet, the rumen succinate concentrations rose 200-fold and rumen propionate concentration decreased. Thus, the plasma succinate concentration can at least partially overcome the effect on gluconeogenesis [14].

The results by Hudman *et al.* [12] indicated that there was an interaction in the animal between Se and phosphate, leading to lower liver Se concentrations (not in blood). Interaction could also occur in the gastrointestinal tract. Consuming fresh grass at pasture or indoor by calves with combined selenium and vitamin E deficiency diet (0.01 ppm Se and 2 mg vitamin E/kg feed) provokes large increases in plasma creatine kinase activity, typical for muscle damage, and up to10-fold higher the percentage of polyunsaturated fatty acids [2]. The increment of Se and Cu in feed leads to reduction of Se in the liver tissue [15]. Antagonism between Se and sulphur can lead to nutritive muscular dystrophy [4]. Kleczkowski *et al.* [15] concluded that this can be corrected by adding simultaneously Zn and Mo to feed.

It is worth to point out that in animals in which the basal diet was selenium-deficient (lower than 0.1 ppm), an addition of inorganic Se to diet produced an increase of the element in the liver, but following the addition of selenite to diets which were already adequate in natural Se, only a small or no increase in tissue Se was observed [35, 39, 40]. An increased dose of Se in lambs diet up to 0.25 ppm, as sodium selenite added to the basal natural diet containing marginally low Se (0,082 ppm), appears to be at or near the Se requirement, as evidenced by the level of selenium in tissue, blood, serum and the maximal tissue GSH-Px activities. In sheep with higher dietary Se levels (0.41 and 0.58 ppm), the increase in the concentration of selenium and in enzyme activity was not so evident [40].

An addition of 1 mg cobalt (per day/sheep) considerably increases the synthesis of vitamin B_{12}. However, an addition of 50 mg cobalt compared with an addition of 1 mg did not result in an increased synthesis of the vitamin [29]. In ruminants, a Co-deficient diet may produce vitamin B_{12} deficiency [19].
At present, one of the most significant problems is to establish the bioavailability of trace elements [26]. Especially important is to know the metabolic synergism of some trace elements. The synergic influence of Se and Co on health and production in sheep is not well known. The aim of the studies was to evaluate the influence of synergic effect when selenium and cobalt heavy pellets were applied together.

MATERIALS AND METHODS

The studies were carried out on 60 three-year-old Suffolk ewes. During the summer, the feed ration was based on pasture forage complemented with barley straw, concentrates and minerals. In the winter season, the animals were fed on maize silage, sugar mangel, and the same roughage. Evetsel preparation (containing 10 mg Se as selenium selenite and 750 mg vitamin E in 10 ml) was given intramuscularly in a dose 5 ml per animal. Cobalt was applied in the form of heavy cobalt granules weight about 12 g (kaolin to Co 1:9). Both trace elements were administered one month before the mating season. Injections of Evetsel were repeated in the 3rd month of pregnancy.

The following four groups were formed, 15 ewes in each group: Se, Co, Se+Co and control. Blood samples were drawn from 6 animals in each group, a month before mating and 2 weeks after the second Se injection (the 3rd

month of pregnancy). Blood metabolic profile of Ht, Hb, glucose, urea, asparpartate aminotransferase, alkaline phosphatase, Ca, P, Mg, Cu and total protein were evaluated using clinical laboratory Bio-tests. The fractions of serum protein were determined using paper electrophoresis method.

The results obtained were statistically analysed; mean values, standard deviations and significane of differences between groups were calculated based on a one-way analysis of variance using the Duncan test.

RESULTS

The effects of selenium and cobalt on reproductive performance of ewes are shown in Table 1. The results indicate that the fertility, prolificacy and the number of twins in the Se group was the highest (87, 131 and 62 %). But a higher number of stillborn lambs (18 and 26 %) and lover weaned rate (100 and 92%) were observed in the groups Se and control respectively. Wherefore, the best production results were achieved by the ewes of the Se+Co group (prolificacy 127% and weaned rate 118%). The rate of conception after the first service of the Suffolk ewes treated with selenium and vitamin E (Evetsel) or cobalt (heavy pellets) preparations were lower (not significantly) than the results obtained for the Se+Co and control groups (73 and 71 vs. 79 and 76 %).

Table 1. The effect of combined application of selenium and cobalt preparations on reproduction of Suffolk ewes [%]

Reproduction rates	Treated			Control
	Se	Co	Se+Co	
1st service conception	73.3	71.4	78.6	75.7
Fertility	86.7	78.6	78.6	75.7
Prolificacy	130.8	120.0	127.3	125.0
Twins	61.5	40.0	42.9	50.0
Weaned rate	100.0^{ab}	100.0^{ab}	118.0^{a}	91.7^{b}
Stillborn	17.6^{ab}	16.7^{ab}	7.1^{b}	26.7^{a}

a,b - p<0.05 in the same rows.

The changes of body weight of ewes during pregnancy and after parturition are presented in Table 2. The average live body weight of animals in the Se and control groups in the 3rd month of pregnancy were significantly (p<0.05) higher (79.0 and 79.4 kg) than in the Se+Co group (73.6 kg). The significantly p<0.05) greatest decreases of body weight gain one month after parturition appeared in the Se and control groups (-11.4 and –11.0 kg) whereas body weight gain in the Se+Co group increased by 0.5 kg.

Table 2. Changes in live body weight of mother ewes during pregnancy and after parturition [kg]

Times of investigation	Treated			Control
	Se	Co	Se+Co	
Body weight				
I before mating	61.3^{b}	62.4^{b}	61.0^{b}	65.1^{a}
II 3rd month of pregnancy	79.0^{a}	77.5^{ab}	73.6^{b}	79.4^{a}
III month after parturition	67.6^{b}	71.1^{ab}	74.1^{a}	68.7^{a}
Body weight gain				
II - I	17.7^{a}	15.1^{ab}	12.6^{b}	14.3^{b}
III - II	-11.4^{b}	-6.4^{ab}	0.5^{a}	-11.0^{b}
III - I	6.3^{b}	8.7^{ab}	13.1^{a}	3.6^{b}

a,b - p<0.05 in the same rows.

During pasturing period (I), before mating (Table 3), the mean levels of urea (11.2 mM//L), AST (45.9 U/L), P inorg. (2.61 mM/L) and beta globulin (9.0 g/L) in the blood serum were significantly higher and albumin levels were significantly lower (24.5 g/L), compared to the stable period data (II) and reference data. It was connected with a higher level of degradable protein in diet during grazing in September. In the stabling period, only the level of glucose in the blood serum (1.34 mM/L) was lower than in the reference data.

Table 3. Mean level of selected blood constituents of Suffolk ewes in the pasturing period before mating (I) and in the stabling period in the 3rd month of pregnancy (II)

Period	Hematocrit (L/L)	Hemoglobin (mM/L)	Glucose (mM/L)	Urea (mM/L)	AST (U/L)	AP (U/L)
I	0.33	7.6	1.91^{xx}	11.2^{xx}	45.9^{xx}	52.7
II	0.35^{xx}	8.2	1.34	7.0	28.9	94.1^{xx}
	Calcium (mM/L)	Phosphorus (mM/L)	Magnesium (mM/L)	Cooper (µM/L)		
I	2.14	2.61^{x}	1.02	10.0		
II	2.54^{xx}	2.23	1.14^{xx}	16.1^{xx}		
	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)			
			alpha 1	alpha 2	beta	gamma
I	59.1	24.5	5.6^{xx}	6.1	9.0^{xx}	13.9
II	59.4	27.0^{x}	4.5	6.8^{x}	6.3	15.0

AST - aspartate aminotransferase, AP- alcaline phosphatase.
x, xx - p<0.05 and p<0.01.

Analysis of the blood serum of ewes Se group in the stabling period (II) in the 3rd month of pregnancy indicated (Table 4) that the levels of glucose, urea, albumin and Mg were significantly higher (p<0.05) and AP and Ca were significantly (p<0.05) lower then in the control group. At the same time in the Se+Co group the levels of blood serum albumin and Cu were the significantly (p<0.05) lower and alfa 2 and beta globulin were the significantly higher.

Table 4. Profiles of selected blood constituents in experimental (Se, Co and Se+Co) and control groups of Suffolk ewes in the 3rd month of pregnancy in the stabling period

Constituents	Treated			Control
	Se	Co	Se+Co	
Hematocrit (L/L)	0.35	0.35	0.33	0.35
Hemoglobin (mM/L)	8.4	8.3	7.8	8.4
Glucose (mM/L)	1.50^{a}	1.31^{ab}	1.51^{a}	1.06^{b}
Urea (mM/L)	8.1^{a}	7.0^{ab}	6.7^{b}	6.3^{b}
Aspartate aminotransferase (U/L)	28.7^{b}	33.4^{a}	27.7^{b}	26.0^{b}
Alcaline phosphatase (U/L)	61.6^{b}	94.7^{ab}	95.5^{ab}	123.5^{a}
Calcium (mM/L)	2.44^{b}	2.73^{a}	2.36^{b}	2.62^{a}
Phosphorus inorganic (mM/L)	2.30	2.30	2.15	2.14
Magnesium (mM/L)	1.20^{a}	1.14^{ab}	1.14^{ab}	1.08^{b}
Cooper (µM/L)	15.6^{ab}	17.6^{a}	12.9^{b}	18.4^{a}
Total protein (g/L)	61.0	61.8	57.7	57.0
Albumin (g/L)	28.8^{a}	28.7^{a}	23.5^{b}	26.9^{ab}
Globulin (g/L): alfa 1	4.7^{ab}	4.0^{b}	5.2^{a}	4.2^{ab}
alfa 2	6.5^{b}	7.0^{ab}	7.3^{a}	6.4^{b}
beta	6.1^{ab}	6.4^{ab}	6.9^{a}	5.6^{b}
gamma	14.9	15.7	14.8	3.9

a, b - p<0.05 in the same row.

DISCUSSION

Reproduction rates

Ramisz *et al.* [28], as well as other authors [11, 23, 24, 30], found that selenium injections significantly influence fertility and prolificacy of ewes. While the first service conception rate of the selenium treated ruminants may have been lower, the level of selenium in blood serum was above marginal value [9]. Gabryszuk [7] stated that injection of vitamin E and Se together did not significantly increase the reproduction and rearing performance in three-year-old ewes with natural optimal level of Se in serum (0.116 µg/ml). For instance, vitamin E had a harmful influence on B-carotene action [25]. The sows that received B-carotene and vitamin E for 21 days following mating had 2.3 piglets less than a B-carotene group, thus, extra supplementation of sows with vitamin E reduced the number of offspring. The conception rate of first service did not significantly increase when cows with high level of protein in diet were treated with heavy cobalt granules [21]. Investigations in livestock suggest [10] that adequate nutritional supply is required for optimal reproduction in both males and females, while additional supplementation seems to have a negative effect.

When plasma selenium deficiency is high, the addition of vitamin E increases fertility. The data of Segerson and Ganapathy [31] demonstrate that supplementation with selenium/vitamin E to adequately fed and maintained ewes, located in geographical areas low in selenium, can result in increased ova fertility (100.0 vs. 77.3 %). This may be explained by the synergic activity of selenium and vitamin E. The synthesis of selenoproteins is greater in Se-deficient sheep. Five selenoproteins in plasma and nine in tissues were detected in selenium-deficient sheep [5] and the selenoprotein P plays a very important role in reproduction function of animals [32].

Changes in body weight of mother ewes

Lipecka *et al.* (17) found a high number of stillborn Suffolk lambs (27.8 %) in ewes of high condition. The live body weight of mother ewes of the experimental Se+Co group, in 3rd month of pregnancy, was the lowest and the number of stillborn lambs was significantly lower. However, body weight gain in the Se+Co group did not decrease one month after parturition.

The ewes lambs treatment combination of vitamin B_{12} injection and selenium given orally or Co and Se supplied in a from of glass bullet, response in body weight and plasma vitamin B_{12} concentration of experimental lambs were less than control groups [33]. In this situation, it should be stressed that there is a significantly higher serum concentration of cobalamine in the Suffolk ewes than in Merino sheep [27]. However, vitamin B_{12} may function in formation of selenium excretion products, thereby reducing an animal's susceptibility to higher selenium concentration [1]. On the other hand, Co above the NRC requirements may aid in digestion of low quality forages [18]. In this way, it is possible that the body weight gain in the Se+Co group one month after parturition will not decrease.

Metabolic profiles

The higher level of degradable protein in diet, in pasturing period (I), before mating, caused the mean levels of urea, inorganic pfosphorus and aspartate aminotransferase in the blood serum was higher than in the reference data.
It seems that metabolic response of Se treated ewes in the 3rd month of pregnancy may have effected in protozoa and celulolytic bacteria enhanced activity, which increased fibre digestibility and protein degradation intensity in the rumen. This way the rumen pH of the Se and Se+Co groups of ewes may have increased and there was alkaline shift in blood, caused by a decrease in Ca content in blood serum [13], whereas higher level of blood serum urea in Se group may have inhibited AP activity [37].

The level of albumin and copper in serum of the Se+Co group was significantly lower than that of the Se and Co groups (Table 4). Almost entire transport of copper in blood is enhanced by blood plasma albumin. If cobalt and selenium are dosed together, the level of copper in liver and in blood may be lower [36]. Intramuscular injection of sodium selenite decreased the level of copper in serum of ewes [3]. Kleczkowski *et al.* [15] concluded that an increase of copper and selenium concentration in feed led to reduction of selenium in liver tissue.
In the blood serum of the Se group of ewes in the 3rd month of pregnancy the levels of glucose, urea, albumin and Mg were significantly higher ($p<0.05$) and AP and Ca were significantly ($p<0.05$) lower then in the control group. At the same time, the levels of blood serum albumin, calcium and copper were significantly ($p<0.05$) lower, and alfa 2 and beta globulin levels were significantly higher in the Se+Co group as compared with the

untreated control group. It is worth to note that the application of selenium and cobalt preparations to mother ewes together affected their reproduction and metabolic serum profiles.

In summary, it is concluded that the fertility, prolificacy and the number of twins in the Se group, treated only selenium with vitamin E, was the highest (87, 131and 62 %). The ewes Se+Co group, treated with selenium and cobalt preparation together, had not only the good fertility and prolificacy (79 and 127%) but the significantly ($p<0.05$) lower body weight in pregnancy and the significantly ($p<0.05$) higher weaned rates compared with control group as well. But conception rate of 1st service of the Suffolk ewes treated with selenium and vitamin E or cobalt (heavy pellets) preparation may be lower than the results obtained in Se+Co and control groups. In blood serum of Se+Co group the level of albumin, Ca and Cu were the significantly ($p<0.05$) lower and alfa and beta globulin were the significantly higher compared with entreated control group. The application of Se and Co preparations together positively influenced the reproduction of the mother ewes and had effect on their metabolic serum blood profiles.

CONCLUSIONS

1. Fertility, prolificacy and the number of twins in the ewes treated with Se and vitamin E (Se group) was the highest.
2. Ewes treated with Se and Co preparation together (Se+Co group) not only had the high fertility and prolificacy, but lower body weight in pregnancy and the highest weaned rates as well.
3. Conception rate of the 1st service of the Suffolk ewes treated with selenium and vitamin E (Evetsel) or cobalt (heavy pellets) preparation may be lower than the results obtained in control group.
4. The level of albumin, Ca and Cu were the lowest and alfa and beta globulin were the highest in blood serum of Se+Co group compared with control group.
5. The application of Se and Co preparations together positively influenced the reproduction of the mother ewes and had effect on their metabolic serum blood profiles.

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Jan Małecki, Aleksandra Balicka-Ramisz
Department of Animal Hygiene and Prophylaxis
Agricultural University of Szczecin
Doktora Judyma 6, 71-466 Szczecin, Poland
Pfone (091) 454-15-21 ext. 330
e–mail: zooh.@demeter.zoo.ar.szczecin.pl

Eugeniusz Malinowski, Kazimierz Supera
Experimental Station of Zootechnical Institute
Warcisława 1, 74-106 Kołbacz, Poland
Phone (091) 312-40-34
e–mail: kolbacz@free.polbox.pl

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