



### ABSTRACT

The present study was conducted to evaluate the effect of parenteral supplementation of cobalt, copper and iron in late pregnancy on ewe hematology parameters and lamb vigour. Twenty ewes were allocated to one of two groups (n=10). In the test group, on day 120 of pregnancy, cobalt, copper and iron were injected at a dose of 0.1 mL/kg BW (Fercobsang, France, cobalt gluconate 5 mg/100 mL, copper gluconate 0.5 mg/100 mL and ferrous citrate 1000 mg/100 mL). Ewes in the control group received equal amounts of normal saline as placebo. Group had no significant effect on ewe hematological parameters (P>0.05). There were no significant effects of parenteral mineral supplementation on lamb birth weight, rectal temperature and weaning weight after birth. Lamb viabilities are reported as scores as these gave a true representation of the effects of treatment on underlying measurements. However, there were no differences between groups in lamb vigour and sucking assistance score. It seems that using of additional trace elements in late pregnancy could be effective only in deficiency situations.

KEY WORDS ewe hematology parameters, lamb viability, late pregnancy, trace elements.

## INTRODUCTION

In the majority of situations some dietary supplementation with major and trace elements is required to prevent deficiencies arising with high producing animals (Church, 1991; Piccione et al. 2007). Sub optimal supply of trace elements and vitamins to the pregnant ewe is a potential risk factor for ewe and lamb mortality. Rooke et al. (2008) concluded from a literature review that evidence was strongest for the involvement of trace elements. Duncan et al. (1981) fed ewes (18 months old) a Co-deficient diet (less than 0.05 mg Co per kg DM) throughout pregnancy and lactation. Control ewes had CoSO<sub>4</sub> added to drinking water and maintained serum vitamin  $B_{12}$  concentration above 1.5 ng/mL. In deficient ewes, serum vitamin B<sub>12</sub> concentrations were always less than 0.5 ng/mL. Lamb

birth weights were normal but all lambs from deficient ewes died before weaning. Numerous studies showed that the administration of iron to calves (orally or parenterally) provided an increase in hematological parameters and a better growth in calves (Bostedt et al. 2000; Mohri et al. 2004; Mohri et al. 2006). On the other hand, there is a report that indicated iron supplementation (injection) had no effect on red blood cell (RBC) parameters and health of supplemented calves (Heidarpour Bami et al. 2008). Positive responses to selenium supplementation throughout gestation have recently been recorded (Munoz et al. 2006; Munoz et al. 2007; Munoz et al. 2008). Similarly, positive lamb responses have been observed in most studies to maternal vitamin E supplementation above requirement in the last third of gestation (Rooke et al. 2008). Supplemental trace elements have inconsistently been reported to improve

ewe productivity and lamb vigour (Merrell, 1999; Rooke *et al.* 2008). Therefore, the aim of this study was to investigate the effects of trace elements supplementally fed during late gestation to ewes on hematology in post parturition and lambs vigour.

# MATERIALS AND METHODS

The study was conducted in a sheep herd with approximately 200 lambs per year at Tehran suburb (Iran). The flock was a semi-intensively managed breeding flock, with both ewes and lambs kept indoors during the suckling period. The experiment was reviewed by the animal's experiments committee of the Agricultural College of Abouraihan, University of Tehran.

Twenty ewes were selected following pregnancy scanning in week 13 of pregnancy. The body condition scores (BCS) of ewes were measured using a scale of 1 to 5 (Jeffries, 1961) and allocated to one of two groups (n=10), which were balanced for age  $(3.98\pm0.69 \text{ year})$  and BCS  $(3.32\pm0.66)$ .

On day 120 of pregnancy, ewes were divided into two experimental groups. In the test group, cobalt, copper and iron were injected at a dose of 0.1 mL/kg body weight of ewes (Fercobsang, France, cobalt gluconate 5 mg/100 mL, copper gluconate 0.5 mg/100 mL and ferrous citrate 1000 mg/100 mL).

Ewes in the control group received equal amounts of normal saline as placebo. The ewes in the two groups were received similar diets during the study. All ewes were offered a basal diet of alfalfa hay (0.5 kg/d) supplemented with 600 g/d of a 160 g/kg crude protein (CP) concentrate from day 105 of gestation until lambing.

All ewes lambed in a straw- bedded pen. Immediately after birth, all lambs received navel treatment with tincture of iodine to prevent joint ill, lambs were tagged and their sex recorded.

Blood samples from ewes were taken from the jugular vein at the beginning of the study (day 0,  $40\pm5$  pre-partum, before injection of trace elements, and saline) and at 24 and 72 h post partum.

Two and half milliliters of blood anticoagulated with disodium-ethylenediaminetetraacetic acid (EDTA) were used for cell blood count (CBC). All tubes were placed immediately on ice and were transferred to the laboratory. Anticoagulated blood was analyzed shortly after collection for measurements of red blood cells (RBC), hemoglobin (Hb), hematocrit (packed cell volume; PCV), and total leukocyte count (white blood cells; WBC) by microhematocrit, cyanmethaemoglobin and standard manual methods, respectively. Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood films (Jain, 1986).

After birth all lambs were observed and the times of each of the following behaviours were recorded for each lamb: shaking of head; on knees; attempting to stand; successfully standing; reaches udder; attempts to suckle; successfully suckles. From these observations, a vigour score was assigned to each lamb according to the progress the lamb had made by 5 min after birth and using the criteria described in Table 1.

 Table 1 Criteria used to assign scores for vigour and sucking assistance to lambs

Score	Description					
Lamb vigour	(5 min after birth)					
0	Extremely active and vigorous lamb, has been or is standing on all four feet					
1	Very active and vigorous lamb, standing on back legs and on knees					
2	Active and vigorous lamb, on chest and holding head up					
3	Weak lamb, lying flat, able to hold head up					
4	Very weak lamb, unable to lift head, little move- ment					
Sucking assis	stance					
0	Lamb sucking well without assistance within 1 h of birth					
1	Lamb sucking well without assistance within 2 h of birth					
2	Lamb given sucking assistance and once or twice in first 24 h after birth					
3	Lamb given sucking assistance, hand-fed colos- trum more than twice, needing help after 1 day old, but able to suck by 3 days old					
4	Lamb still needing help to suck when more than 3 days old					

Scores for sucking assistance were also assigned to each lamb from the criteria also listed in Table 1 (Rooke *et al.* 2009).

At 2 h after birth rectal temperature and body weight of each lamb were recorded. Ewes and their lambs were turned out to grazing approximately 3 days after birth. All lamb deaths and lamb weights at weaning (14 weeks of age) were recorded.

The procedure mixed program of SAS (2004) was used to analyze the measurements. Because ewe blood parameters were measured over the time, a repeated measures approach using ANOVA with mixed linear models in SAS 9.1 was used. The means were compared by the Duncan's ttest. P < 0.05 was considered as significant.

# **RESULTS AND DISCUSSION**

The results are summarized in Tables 2 and 3. Group had no significant effect on ewe hematological parameters (P>0.05). Sampling time had no significant effects on the most measured parameters except PCV, RBC, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).

Parameters	G	Group		Effects (P-value)		
	Control	Test	SEM	Group	Sampling time	Group × sampling time
PCV (%)	34.09	35.02	0.54	0.23	0.006	0.74
RBC (10 <sup>12</sup> /L)	9.99	10.52	0.22	0.09	0.0006	0.50
Hb (g/L)	113.2	109.5	6.54	0.13	0.32	0.56
MCV (fl)	34.16	33.46	0.35	0.17	0.02	0.09
MCH (pg)	11.34	11.21	0.05	0.08	0.007	0.25
MCHC (%)	33.18	33.63	0.27	0.25	0.07	0.07
WBC (10 <sup>9</sup> /L)	10.31	9.68	0.58	0.39	0.13	0.29
Neut (10 <sup>9</sup> /L)	6.45	6.34	0.65	0.32	0.14	0.28
Lymph (109/L)	3.26	3.11	0.21	0.14	0.09	0.31
Mono(10 <sup>9</sup> /L)	0.35	0.20	0.12	0.37	0.95	0.30

Table 2 Effect of parenteral trace elements supplementation on ewe hematology parameters

PCV: packed cell volume; RBC: red cell count; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell count; Neut: neutrophil; Lymph: lymphocyte and Mono: monocyte.

SEM: standard error of the means.

Significant interactions between sampling time and group were not seen for any of measured parameters (P>0.05). There were no significant effects (Table 3) of parenteral mineral supplementation on lamb birth weight, rectal temperature and weaning weight after birth. Lamb viabilities are reported as scores (Table 3) as these gave a true representation of the effects of treatment on underlying measurements. However, there were no differences between groups in lamb vigour and sucking assistance score. Overall, two lambs of the control group and one of the test group died after birth.

 Table 3
 Effect of treatment groups on lamb vigour, sucking assistance scores and lamb performance

Donomotono	Gro	up	SEM	P-value
Parameters	Control	Test	SEM	
Body temperature (°C)	39.20	39.00	0.55	0.31
Lamb vigour	1.07	1.06	0.05	0.89
Sucking assistance	0.87	0.81	0.06	0.67
Birth weight (kg)	3.83	4.00	0.44	0.28
Weaning weight (kg)	18.65	18.95	3.01	0.77
Lamb mortality	2	1	-	-

SEM: standard error of the means.

#### Hematology of ewes

As it was noted, no difference was observed between the groups for the hematology parameters. No data in this contest were found in the available literature for ewes. Most studies focused on hematology of newborn lamb and calves as various studies reported that administration of iron and copper provided an increase in RBC parameters and MCV in calves (Bostedt *et al.* 2000; Mohri *et al.* 2004; Heidarpour Bami *et al.* 2008).

Bostedt and Schramel (1982), Bostedt *et al.* (1990) and Zumbo *et al.* (2011) reported that a progressive reduction in serum iron concentration, RBC, and Hb occurs over the first weeks of life and administration of iron and copper provided an increase in RBC parameters and MCV in calves (Bostedt *et al.* 2000; Mohri *et al.* 2004; Heidarpour Bami *et al.* 2008). Deficiencies are most likely to occur in young animals because milk is low in iron (about 10 ppm; Heidarpour Bami *et al.* 2008). Also a similar trend was reported for MCV in newborn animals as Heidarpour Bami *et al.* (2008) reported that newborns of domestic animals have large erythrocytes at the birth, and then over the first weeks of life, the volume of erythrocytes reduces and results in reduction in MCV. It seems that in adult animals that receive sufficient amounts of dietary iron and copper, administration of supplemental trace elements could not be useful and does not change the hematological parameters as observed in this study.

#### Lamb performance and vigour

Treatment had no effect on lamb birth and weaning weights. There were a few studies that reported significant effects of supplemental cobalt on performance of lambs by administration of this trace element in late pregnancy of ewes. However in most of these studies cobalt supplementation was applied to ewes that were fed deficient diets. Fisher and MacPherson (1991) reported lamb birth weights did not differ due to application of cobalt supplementation in late pregnancy of ewes, but pre weaning losses were significantly reduced in the supplemented group. In this study, time to find the udder and suck was reduced by supplementation. It seems that cobalt deficiency reduced lamb viability and both early and late supplementation partially ameliorated the deficiency compared to supplementation throughout gestation. Rooke et al. (2008) concluded that maintaining pregnant ewes on cobalt-deficient diets leads to adverse effects on lamb performance prior to weaning and supplementation remedies this. These authors in a comprehensive review reported that there were no experimental evidences for improvement in lamb status by cobalt supplementation of the ewe when the diet consisted of cobaltadequate ewes.

In agreement with our results, Van Niekerk *et al.* (1995) reported no significant effects of supplementing of copper

on birth weight or weaning weight. There is one piece of evidence for a positive response to copper, practically, as long as deficiency is avoided, copper supplementation would not be advised because of the risks of inducing toxicity (Rooke *et al.* 2008).

Milk fed lambs are most at risk of anemia because milk iron concentrations are low, although if the ewe herself is adequate in iron, then lamb liver iron stores are likely to be sufficient to buffer the lamb until weaning (Rooke et al. 2008). No reports have been found describing the effects of iron status on lamb viability or related topics. However, one should remember that iron deficiency in rodents affects placental development (Mc Ardle et al. 2006). To study of viability of newborn lambs, a further issue is the sensitivity of the scoring system used to measure lamb vigour. The lamb vigour score was derived from underlying measurements such as time for the lamb to stand, contact the udder and suckle. Fisher and Mac Pherson (1991) based reports of improved lamb vigour on these underlying measurements. However, there were no differences in time to stand, contact the udder and suckle the ewe etc. in the present experiment.

# CONCLUSION

In conclusion, this experiment did not find any responses in lamb vigour or performance to supplementation of the ewe with cobalt, copper and iron in late pregnancy of ewes. Also supplementation of these trace elements had no significant effects on hematology parameters in ewes. It seems that using of additional trace elements in late pregnancy could be effective only in deficiency situations.

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