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Trace Minerals in Blood and Colostrum in Naemi Ewes and their Neonates Fed with Long Term Prepartum Sustained-Release Trace Elements Ruminal Bolus

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Authors' Contribution
MMA did all experimental work, wrote the manuscript and followed up the team. RSA contiburted to the field work and analysed the data and results. MA did the lab work for milk and wrote the relevant part in the manuscript. SN analysed the blood samples. RSA and SN helped in writing and improving the manuscript.

Key words
Trace minerals boluses, Naemi ewes, Newborns, Colostrum, Metabolites.

ABSTRACT

The aim of this study was to find the effect of the administration of slow release boluse of trace elements bolus in Naemi ewes during late gestation. Thirty Naemi ewes were divided into two groups. One group served as a control while the other was treated with rumen bolus of trace elements containing copper (Cu) cobalt (Co); selenium (Se), manganese (Mn); zinc (Zn) and iodine (I). Blood samples were collected from ewes and their newborns at parturition. Colostrum yield and biochemical analysis at parturition was also determined. A significant ($P<0.05$) increase of calcium (Ca), Zn, Co and Se levels in blood of ewes treated with bolus with a significant increase in phosphorous (P), Co and Se in the blood of their newborn lambs. Furthermore, a significantly higher ($P<0.05$) inorganic matter percentage in colostrum was found in treated group compared to the control. The body weight of lambs in treated group at 30 and 60 days was significantly ($P<0.05$) higher when compared with the control. In conclusion, trace mineral boluses supplementation to pregnant ewes at late gestation improved minerals status of the ewes and their newborns at parturition and consequently enhanced body weight.

INTRODUCTION

Dam nutrition is a crucial factor during the late pregnancy for the survival and health status of the newborn in small ruminants (Mahoub *et al*., 2013). For the proper development of newborn, an adequate supply of nutrients is essential. It has been documented that inadequate supply of essential nutrients may negatively affect the birth weight and health status of newborn (Abdollahi *et al*., 2013). In addition, suboptimal nutrition also retards the growth of mammary gland, lactation performance leading to reduced colostrum and milk availability of for the newborn (Tygesen *et al*., 2008). The negative effects of malnutrition in newborns are more prominent where there is scarce availability of feed stuffs (Alhidary *et al*., 2016a). The low growth of the forages is usually associated with inadequate supply of trace minerals which are necessary for the proper growth and development of the newborns (Mundell *et al*., 2012).

Trace minerals have been identified as essential for the proper growth of farm animals (Alhidary *et al*., 2016b, c). The trace elements required by small ruminants include copper (Cu), manganese (Mn), selenium (Se), molybdenum (Mo), iodine (I), cobalt (Co), iron (Fe), chromium (Cr), nickel (Ni), and zinc (Zn) (Abdelrahman *et al*., 2017). It has been well documented that supplementation of trace minerals improve productive and reproductive performance in ruminants (Mundell *et al*., 2012; Abdollahi *et al*., 2013; Abdelrahman *et al*., 2017). Due to inadequate supply of essential trace elements, supplementation of long acting rumen trace element bolus is considered important for the alleviation of the minerals deficiency (Alhidary *et al*., 2016a, b). Deficiency of trace minerals causes metabolic disorders, impaired fetal growth, stillbirth, abortion, early embryonic death and increase the mortality rate in newborn (Hostetler *et al*., 2003; Abdollahi *et al*., 2013).

This study was planned to investigate the effect of using trace minerals boluses as a source of Se, Cu, Zn, Co and Mn to Naemi ewes at late gestation (60 days prepartum) on the mineral profile of the ewes and their newborn lambs at birth, colostrum composition and weight of the newborn.

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MATERIALS AND METHODS

All ewes and newborn lambs used in this trial were treated according to King Saud University regulations for animal care, handling and management.

Animals and management

A total of thirty healthy multiparous ewes of Naemi breed (3-4 years old; single lambing) were selected at late gestation (-60 days prepartum) at Al- Khaldiah farm located in the semi-arid area in Al- Riyadh region, Saudi Arabia. The ewes were raised under an intensive production system having identical conditions of feeding and management. Randomly half of the ewes were treated with a ruminal trace mineral bolus. Each bolus contains copper 3,944 mg; cobalt 95 mg; selenium 45 mg; manganese 3013 mg; zinc 4366 mg and iodine 330 mg. The bolus lies in the reticulum and dissolves from a constant surface area and provides a continuous and regular supply of the above trace minerals for 180 days. The other 15 ewes were considered as a control group. The experiment lasted for 60 days postpartum.

The ewes were fed pelleted concentrate diet (450 g per head; pellets consists of barley (*Hordeum vulgare*), corn maize (*Zea mays*), soybean hull (*Glycine max*), palm (*Elaeis guineensis*) seed meal, alfalfa (*Medicago sativa*), sugar molasses and Rhodes grass (*Chloris gayana*) (750 g per head; Table I). Water was provided *ad libitum* to all ewes for the entire period. Newborn lambs from both groups were weighted at birth, 30 and 60 days old.

Samples collection and preparation

Blood samples were collected from the jugular vein at parturition from the ewes and their newborns using vacutainer tube without anticoagulant to separate serum. Serum was separated by centrifuge (3000 rpm during 15 min). Colostrum samples were collected from each ewe at the second day postpartum, morning and evening, and homogenized. Serum and colostrum samples were stored at -20ºC till analysis.

Biochemical analysis

Serum samples were analyzed for glucose, total protein, cholesterol, albumin, Cu, Zn, Ca and P by spectrophotometer using commercial kits (United diagnostics Industry, Dammam 31413, KSA). Selenium concentration was analyzed using ICP-MS analysis. Moreover, colostrum samples were analyzed for fat, protein, lactose, total solid (TS) and solid not fat (SNF) values using Milko Scan (Minor Type 78100, Foss Electric, Denmark).

Table I.- Chemical composition of the experimental concentrate pellets and Rhodes grass (As fed).

Nutrient	Concentrate pellets	Rhodes grass
Crude protein%	12.99	9.77
Energy (Mcal/kg)	2.95*	4.14**
Crude Fiber%	10.11	32.10
Crude fat%	2.11	1.66
Inorganic matter%	7.62	12.10
Calcium%	1.51	-
Phosphorus%	0.54	-

*, Metabolizable energy; **, Gross energy.

Statistical analysis

All data were analyzed by analysis of variance using the GLM procedure (SAS, 2009) as a complete randomized design (CRD) with repeated measurements. The differences between mean were tested by protected least significant difference. P values less than 0.05 was statistically considered significant.

Table II.- The effect of mineral supplementation at late gestation of Naemi ewes on their minerals and newborn status.

Parameters	Phosphorus (mg/dl)	Calcium (mg/dl)	Zinc (mg/dl)	Copper (mg/dl)	Cobalt (µg/dl)	Selenium (Ppm)
Newborn lambs						
Control	3.84	11.06	0.29	0.239	0.68	0.03
Treated	8.50	12.4	0.34	0.385	1.10	0.06
SEM	1.01	1.49	0.02	0.03	0.02	0.001
P value	0.001	0.08	0.11	0.09	0.04	0.03
Ewes						
Control	6.16	6.22	0.281	0.298	0.92	0.03
Treated	6.88	13.53	0.370	0.313	1.62	0.05
SEM	1.03	1.84	0.06	0.04	0.03	0.003
P value	0.44	0.001	0.03	0.23	0.03	0.02

Table III.- The effect of mineral supplementation at late gestation of Naemi ewes on their metabolites and newborn status.

Parameters	Glucose (mg/dl)	Total protein (g/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	Urea N (mg/l)	Triglyceride (mg/l)
Newborn lambs						
Control	53.85	10.45	127.25	0.69	99.4	84.65
Treated	30.08	8.67	118	0.64	81.87	60.03
SEM	8.66	0.95	10.79	0.04	5.48	8.62
P value	0.03	0.32	0.12	0.87	0.023	0.031
Ewes						
Control	34.3	7.11	55.67	0.65	82.23	65.10
Treated	39.5	9.58	111.40	0.76	72.03	47.30
SEM	4.18	0.86	5.77	0.052	6.22	3.67
P value	0.98	0.04	0.03	0.22	0.12	0.98

Table IV.- Effect of trace mineral boluses on colostrum composition of Naemi ewes raised under intensive system.

Nutrients	Control	Treated	SEM	P values
Colostrum yield (L/d)	1.33	1.41	0.14	0.34
Fat (%)	5.83	5.70	0.315	0.532
Protein (%)	5.07	4.56	0.215	0.289
Lactose (%)	4.69	4.64	0.077	0.781
Total solid (%)	15.66	16.01	0.421	0.991
Solid non fat (%)	11.03	11.04	0.179	0.730
FPD (%)	0.63	0.61	0.009	0.730
Inorganic matter (%)	0.90	1.08	0.056	0.05

RESULTS

A significant increase of Ca, Zn, Co and Se levels in blood of ewes treated with boluses was observed when compared to the control (Table II). For the newborn lambs, P, Co and Se were significantly increased in the blood of lambs in the boluses treated ewes. In dams, Ca, Zn, Co and Se were significantly ($P<0.05$) high in the treated groups.

Blood glucose, total protein, cholesterol, creatinine, urea-N and triglyceride levels of Naemi ewes and their newborn lambs in both control and bolus treated groups are reported in Table III. A significant ($P<0.05$) increase in total protein and cholesterol level in blood of ewes treated with bolus when compared to the control. For the newborn lambs, glucose, urea-N and triglyceride were significantly ($P<0.05$) decreased in the blood of lambs from the bolus treated ewes. The nutritive values of colostrum in both groups were reported in Table IV. The colostrum inorganic matter was significantly affected by bolus supplement compared with unsupplemented ewes (1.08 *vs* 0.90%: $P < 0.05$). The total solid in treated ewe colostrum was numerically higher compared with the control (16.01 *vs* 15.66%). Lambs in treated ewes with boluses showed a significantly ($P<0.05$) higher body weight at 30 and 60 days of age when compared with lambs from the control group (Fig. 1).

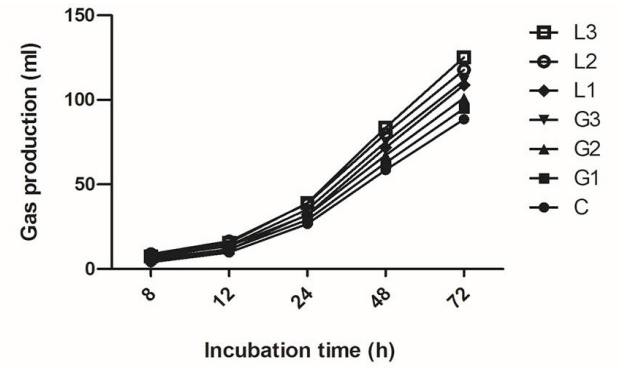

Fig. 1. Effect of trace mineral boluses on the body weight of Naemi lambs at birth, 30 and 60 days old.

DISCUSSION

Late gestation and early lactation are the most critical stages in ewes' life cycle. The daily nutrient requirements increase for fetal rapid growth and milk production. All mineral values in blood of dams and the newborn fell within the normal recommended range, except for Co and Zn (Puls, 1994; Herdt and Hoff, 2011). Phosphorus concentration in blood of the newborn lambs was significantly higher than the control, but did not vary between the ewes. These values agreed with those reported by Antunovic *et al*. (2011) and Azab and Abdel-Maksoud (1999). The opposite trend was reported for the Ca levels in which ewes from the control group showed a significantly lower level compared with the treated ewes, but no significant difference was reported for the

newborn from both the group. These values agreed with that reported by Antunovic *et al*. (2011) and Yildiz *et al*. (2005) in lactating ewes at early lactation.

The Se level in blood was above the lower limit (0.020 ppm) recommended by NRC (1985) and Underwood and Suttle (1999). The blood Se concentration of supplemented ewes with the mineral boluses was significantly increased compared with the control. The same trend was reported for newborn lambs. The trace minerals boluses were found to be very effective to maintain an adequate supply of Se during critical pregnancy period and lactation. This finding completely agreed with the results reported by Hayashida *et al*. (2003).

Copper concentration in blood of ewes supplemented with trace mineral bolus and their newborns were numerically higher compared with the control group. Both groups showed a higher Cu concentration compared with the reference range reported by Underwood and Suttle (1999) and Puls (1994) for ruminants. The levels reported in this study were below the toxic levels. Hayashida *et al*. (2003) reported a significant increase in plasma Cu concentration of does supplemented with trace mineral boluses which partially agreed with our findings. The same trend was reported by Abdollahi *et al*. (2013) when supplemented ewes with trace mineral slow release boluses. Results indicated that trace mineral boluses improve the Cu status of pregnant ewes at late gestation and their newborn during the early lactation periods.

A significant increase of Zn and Co levels in blood of treated ewes with bolus were expected due to the presence of high level of both minerals. A numerical increase in Zn was reported in the newborns of the treated group, while Co level was significantly high in the newborn compared with the control. Cobalt level was higher than normal even in the control group (0.10 - 0.30 µg dl^{-1}; Miller *et al*., 1993). Abdelrahman *et al*. (2001) reported a higher Co concentration (0.49 -1.30 µg dl^{-1}) in blood of ewes and their newborn raised under the intensive system which agreed with our findings.

The reference range of Zn in the blood of ewes ranged from 0.08 to 0.12 mg dl^{-1} according to Puls (1994) and Herdt and Hoff (2011). Zinc concentration for ewes and newborns from boluses treated group were higher than the normal range and compared with the control group. These values were below the toxic levels reported by Puls (1994).

As a general trend regarding the effect of trace mineral supplementation, Kendall *et al*. (1997) reported an elevation in blood Zn, Se and Co status as a result of trace minerals bolus supplementation which completely agreed with our findings.

The reference range of protein, Urea-N, cholesterol, triglyceride, creatinine and total protein are as follow: 50.09 - 80.00 mg/dl; 8.01- 20.00 mg/dl; 52.12 - 76.10 mg/dl; 9.0 - 17.600 mg dl^{-1}; 0.57-1.23 mg dl^{-1} and 6.0-7.90 g dl^{-1} g/dl, respectively (Antunovic *et al*., 2011; Hindson and Winter, 2002). The values for total protein and creatinine were falling within the reference range, but glucose values were lower for dams and newborn lambs in the treated group (Table III). The low glucose levels at parturition may be associated with the high demand of glucose for fetal development at late gestation, which negatively affects the glucose levels of ewes at parturition. Furthermore, a higher concentration of urea-N, cholesterol and triglyceride in the blood of the control and treated ewes may be due to the higher demand of energy which resulted in breakdown of protein and fat reserve of the body.

Impact of trace minerals supplementation on milk yield and composition are variable. Nocek *et al*. (2006) and Siciliano *et al*. (2008) reported a significant effect of trace minerals supplementation on milk components which generally agreed with our findings. Changes in milk composition could be due to the involvement of these trace minerals in many other physiological processes (McDowell, 2003). On the other hand, Cortinhas *et al*. (2012) reported no effect of Zn, Cu and Se source on milk yield and composition.

Minerals have been found to be very crucial in ruminants' growth and productivity (Underwood, 1981). Minerals required for proper functioning of ruminants' body tissues and prevent diseases as a negative effect of their deficiency (Langlands *et al*., 1994; Swecker *et al*., 1995). Minerals involve in the defense system against free radicals that damage the biological system through the formation of the metalloenzymes which include glutathione peroxidase, catalase and superoxide dismutase (McDowell, 2002).

There were few studies that reported the effect of trace minerals supplementation on birth and weaning weight by administration of these trace minerals in late pregnancy of ewes. Norouzian *et al*. (2014) and Fisher and MacPherson (1991) reported that birth weight did not change in response to Co supplementation in late pregnancy in ewes which is in agreement with our results. The same trend was reported by Van Niekerk *et al*. (1995) when pregnant ewes supplemented with Cu at late gestation. The results of this trial may be due to the optimum level of Zn, Se and Co status of the newborns after birth and the corresponding colostrum yield.

CONCLUSION

Using trace minerals slow release boluses supplementation to Naemi ewes improved Se, Zn and Co status of ewes at parturition and Co and Se of their

newborns at birth. Inorganic percentage in colostrum increased in treated group and consequently weight of neonates.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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