Indian Journal of Animal Sciences **87** (9): 1095–1096, September 2017/Short communication

Effect of peripartum micronutrient supplementation on postpartum udder health in high-yielding crossbred cattle

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Received: 9 September 2016; Accepted: 3 April 2017

Key words: Crossbred cattle, High yielding, Peripartum, Postpartum, Udder health

Supplementation of micronutrients around peripartum period have shown to increase disease resistance as well as reduce the incidence of clinical mastitis in cattle by increasing the activity of neutrophils and lymphocytes both *in vivo* and *in vitro* (Dang *et al*. 2013, De *et al*. 2015). But, all these studies stated earlier focused only on blood lymphocytes and neutrophils. Till date, very limited literatures are available on the effect of periparturient micronutrient supplementation on postpartum udder health. Therefore the present study was intended to find out the effect of micronutrient supplementation on udder health in terms of *in vitro* activity of milk leukocytes (phagocytic activity of milk neutrophils and milk lymphocyte proliferation response) in high-yielding crossbred cows.

High-yielding crossbred cows (12; daily yield 8.5 kg/day) of third parity in late pregnancy were selected for the present study. The animals were divided into 2 groups viz. supplemented (6) and unsupplemented (6). Supplemented animals were supplied with a mineral mixture (@25 g daily in the morning) over and above the control feeding from 45 days prepartum to 45 days postpartum (based on their expected date of calving). The mineral mixture was formulated as per NRC recommendations (NRC 2001) and contained copper (10 mg/kg dry matter), selenium (0.3–0.4 ppm in total ration), manganese (13 mg/kg dry matter) and zinc (43 mg/kg dry matter). Milk samples (200 ml) were collected from each animal through hand milking on 7th, 30th and 90th day of lactation after proper washing and teat dipping with disinfectant solutions. Somatic cell counts (SCC) of milk samples was evaluated microscopically. *In vitro* phagocytic index (PI) of milk leukocytes was performed colorimetrically by nitro blue tetrazolium (NBT) assay (Choi *et al*. 2006) for milk neutrophils and MTT assay (tetrazolium) assay (Mosmann 1983) for milk lymphocyte. All analyses were done using two-way ANOVA considering group and postpartum days as factors by SYSTAT software package.

The milk somatic cell count, phagocytic index of milk neutrophils and *in vitro* lymphocyte proliferation response during different days of post-partum in supplemented and unsupplemented group of high-yielding crossbred cows are presented in Table 1. There was significantly (P<0.05) lower milk somatic cell count in supplemented group of cows compared to the unsupplemented group. Milk somatic cell count differed significantly (P<0.05) between different days

Table 1. Milk SCC, phagocytic index of milk neutrophils and *in vitro* lymphocyte proliferation response during different days of post-partum in supplemented and unsupplemented group of high-yielding crossbred cows

Parameter	Group	Days postpartum		
		7	30	90
Milk SCC	Supplemented	$2.50^{a}\pm0.51$	$1.85^{a}\pm0.34$	$1.60^{ab}\pm0.32$
	Unsupplemented	$3.10^{x}\pm0.44$	$2.92^{x}\pm0.38$	$2.17^{y}\pm0.43$
In vitro phagocytic index of milk neutrophils	Supplemented	$0.37^{a}\pm0.108$	$0.19^{b}\pm0.102$	$0.16^{b}\pm0.102$
	Unsupplemented	$0.30^{a}\pm0.08$	$0.14^{b}\pm0.08$	$0.22^{c}\pm0.08$
In vitro milk lymphocyte proliferation response	Supplemented	$1.20^{a}\pm0.19$	$1.00^{a}\pm0.18$	$1.30^{a}\pm0.18$
	Unsupplemented	$0.70^{x}\pm0.15$	$0.90^{ay}\pm0.15$	$1.10^{axy}\pm0.15$

Values are expressed as mean±SE. Values with a different superscript differed significantly (P<0.05) [^{a,b}between groups, ^{x,y}between days)].

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of post-partum in both the group of cows.

Supplementation of micronutrients particularly Se (Moeini *et al*. 2009) and Zn (Kellogg *et al*. 2004) have been shown to lower the milk SCC reported earlier is reflected in our investigation also. It may be due to the fact that micronutrients, particularly Zn acts as an epithelialization promoting agents (Spears 2000)

In vitro phagocytic activity of milk neutrophils did not differ significantly between the two groups as it varied significantly ($P<0.01$) among different days of post-partum. There was a significant ($P<0.05$) higher activity of milk lymphocytes in supplemented groups compared to un-supplemented groups.

Decreased phagocytic ability of blood neutrophils was reported in Cu (Spears 2000) and Zn deficiency (De *et al*. 2015). In a related study, Dang *et al*. (2013) also reported increased phagocytic activity of blood neutrophils upon micronutrient supplementation around peripartum period. The beneficial effects of micronutrients on neutrophil function may be due to their protective effect on phagocytic cells from autoxidative damage during the respiratory burst, leakage of free radicals from the phagolysosomes, or failure to detoxify these products which could affect the microbicidal and metabolic functions of phagocytic cells (Vallee and Falchuk 1993).

Micronutrients particularly Cu and Zn had been reported to increase all aspects of lymphocyte functions including cell proliferation and replication (Tomlinson *et al*. 2008) together with antibody and cytokine production. McKenzie *et al*. (1998) also stated that Se is essential in helping leukocytes reduce the formation of peroxides.

The present investigation permitted us to conclude that peripartum micronutrient supplementation (from 45 days prepartum to 45 days postpartum) significantly increase the udder immunity indicated by decreased milk SCC and increased phagocytic activity of milk neutrophils and lymphocyte proliferation thus reducing the chances of intramammary infections and mastitis.

SUMMARY

The study was aimed to evaluate the effect of peripartum micronutrient supplementation on postpartum udder health in high-yielding crossbred cows. High-yielding crossbred cows (12) of late pregnancy were selected and divided into two groups, viz. supplemented (6) and unsupplemented (6). Supplemented animals were supplied with a mineral mixture (@25 g daily) fortified with copper (Cu), selenium (Se), manganese (Mn) and zinc (Zn) as per the recommendation of National Research Council (NRC) over and above the normal ration starting from 45 days precalving and continued till 45 days postcalving (based on their expected date of calving). Milk samples from each animal were collected and different attributes of udder immunity were evaluated. The supplemented group exhibited higher udder immunity as depicted by lower milk somatic cell count and higher activity of milk lymphocytes.

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