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Research article

Dietary organic trace mineral supplement affects steroid hormone, antioxidant enzyme concentrations, and reproductive performances in dairy cows

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Abstract

Supplementation of organic trace mineral (OTM) may affect reproductive functions in several aspects and has been studied, but very rare is reported in dairy cows. To determine the effects of dietary OTM supplement during pre- and postpartum with timed artificial insemination (TAI) protocol on steroid hormones, antioxidant enzymes, and reproductive performances in dairy cows. Prepartum Holstein cows (n = 60) were randomly assigned to received either a control diet or OTM supplemented for 42 days. Both groups were fed the same total mixed ration, but were supplemented with 5 g/head/d OTM (Bioplex®) in the treatment group. Blood and follicular fluid samples were collected to determine progesterone (P4), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and estradiol (E2) concentrations. Cows fed OTM had better placental expulsion period, milk yield, and reproductive performance parameters (day to first ovulation and estrus, percentage of first estrus and size of preovulatory follicle) than control cows ($P < 0.05$). Cows fed OTM affected ($P < 0.05$) the serum concentration of P4 on days 12, 15, 18, 21, and 42 after TAI. Follicular fluid concentrations of E2 in large follicle in OTM cows were greater than control cows (336.3 and 376.9 ng/mL; $P < 0.05$). Cows supplemented with OTM had greater serum SOD and GSH-Px activities than control cows (15.5 vs. 10.6 U/mL and 12.1 vs. 9.9 U/mL; $P < 0.05$). In conclusion, supplements with OTM improved uterine and placental health, steroid hormones concentrations, and antioxidant enzyme activities but did not improve conception rate in dairy cows.

Keywords: Antioxidant enzymes, Conception rate, Dairy cow, Organic trace minerals, Steroid hormones

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INTRODUCTION

Higher nutrient requirements for parturition and milk production by postpartum dairy cows cause reduced feed intake, leading to negative energy balance (Roche et al., 2009). Postpartum uterine disorders including metritis, endometritis, and retained fetal membrane (RFM) decreased milk production and reproductive performance (Bicalho et al., 2014). Uterine infections caused low fertility by interrupting uterine and ovarian function after parturition in dairy cows (Sheldon et al., 2009). The decrease in conception rate and fertility are caused by inflammatory mediators acting on the hypothalamus, pituitary, and ovary. The lipopolysaccharide of inflammatory cytokines secretion can suppress the release of gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) associated with slower growth of the ovarian follicle and decreased estradiol (E2) secretion, which could reduce the ability to ovulate in postpartum cows (Battaglia et al., 2000).

Trace minerals are required for functioning of enzymes involved in the antioxidant defense system and affect immune cells via mechanisms distinct from antioxidant properties (Bicalho et al., 2014; Machado et al., 2014). Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are crucial enzymes playing a vital role in protecting cells from reactive oxygen species (ROS) in the oxidant system (Machado et al., 2014). Trace mineral product containing zinc (Zn), copper (Cu), and selenium (Se) supplementation has been associated with higher antioxidant capacity of SOD and GSH-Px, resulting in reduced oxidative stress, protecting both extracellular and intracellular cell membranes, and improved uterine health after parturition. Moreover, Se stimulates the estradiol production by bovine granulosa cells (Basini and Tamanini, 2000; Cerny et al., 2016). Thus, trace minerals are essential for growth, health, fertility, productive performance and fertility in dairy cows (Machado et al., 2013). Sources of OTM (complexes, proteinases and amino acid chelates) have been reported as having greater bioavailability and retention in the digestive tract than inorganic forms (Cortinhas et al., 2010; Formigoni et al., 2011). Although the bioavailability of OTM has been inconsistent in the literatures, multiple reports have demonstrated the significant reproductive improvement in animals supplemented with the OTM (Nocek et al., 2006; Griffiths et al., 2007; Formigoni et al., 2011).

We hypothesized that OTM supplement during pre- and postpartum in maternal diet with timed artificial insemination (TAI) using a modified Ovsynch protocol will affect concentrations of steroid hormones, antioxidant enzymes, and conception rates in dairy cows. Therefore, the objective of this experiment was to determine effects of dietary OTM supplements during pre- and postpartum with TAI using a modified Ovsynch protocol on steroid hormones, antioxidant enzymes, and reproductive performances in dairy cows.

MATERIALS AND METHODS

Animals and experimental design

All experimental procedures were managed according to the guidelines approved by the Animal Ethics Committee of Khon Kaen University and the license no. of 1-04051-2559. This study was performed at a semi-commercial dairy farm located in Khon Kaen province. Prepartum Holstein dairy cows (n = 60) were blocked by lactation number (lactation 1-3 vs. ≥4) before being randomly assigned into 1 of 2 groups (Figure 1): control (no supplemental OTM in diet; n = 30) or OTM supplemented (n = 30). In OTM supplemented group, cows were supplemented with 5 g/head/day OTM (Bioplex®; Alltech Inc.), beginning at 21 days (-21 days) prior to expected calving date and for 21 days after parturition. Five grams of Bioplex® containing 360 mg of Zn, 100 mg of Mn, 110 mg of Cu, 10 mg of Co, and 3 mg of Se. All cows were fed the same total mixed ration (TMR), formulated to meet the nutrient requirements according to NRC (2001) as shown in Table 1.

Table 1 Ingredient composition of the diet[1]

Item	Diet	
	Prepartum	Postpartum
Ingredient, % of DM		
Chopped rice straw	25.5	20.0
Cassava ship	40.5	35.0
Dried brewer's grain	11.0	15.0
Soybean meal (41.6% CP)	10.0	16.5
Ground corn	11.0	11.5
Urea	1.5	1.5
Vitamin premix	0.5	0.5
Chemical composition, % of DM		
CP	13.5	16.8
NDF	27.2	26.3
ADF	15.5	14.8
TDN (calculated)[2]	61.7	63.7

CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; TDN = total digestible nutrient.
[1] The TMR had a forage-to-concentrate ratio of 40:60 (DM basis) with the forage ratio containing 40.5 or 35.0% Chopped rice straw and 25.5 or 20.0% Cassava ship.
[2] Calculated using NRC (2001) guideline.

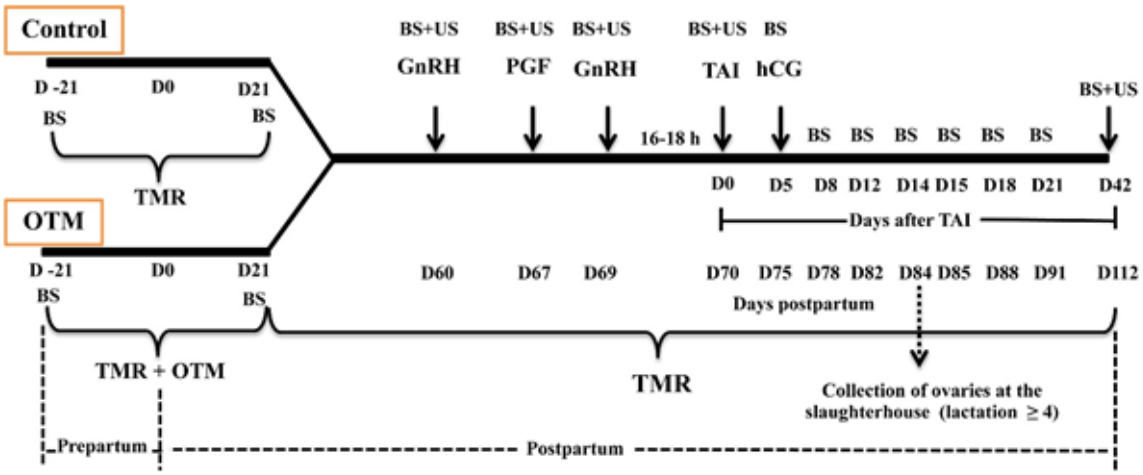

Figure 1 Flowchart of an experiment design indicating dietary treatment, modified Ovsynch protocol with timed artificial insemination (TAI), and sample collection. TMR = total mixed ration, OTM = organic trace mineral (Bioplex®), BS = blood sample, US = ultrasonography, GnRH = gonadotropin releasing hormone (Receptal®), PGF = prostaglandin F2α (Lutalyse®), hCG = human Chorionic Gonadotropin (Choluron®).

At 60 days postpartum, all cows were subjected to a modified Ovsynch protocol (Figure 1). Cows were received with 10 µg of GnRH agonist (Buserelin, Receptal®, Intervet, Auckland, New Zealand), followed 7 days (day 67) later by an injection of 25 mg PGF2α (Lutalyse®, Pfizer Animal Health, New York, USA), the second injection of 10 µg of GnRH agonist was administered at 48 h later (day 69) to all cows, and TAI approximately 16 – 18 h (treated as day 0) after the second GnRH injection. All cows were received 1,500 IU of hCG (Choluron®, Pfizer Animal Health, New York, USA) on day 5 after TAI as described (Navanukraw et al., 2015).

Parturition scores were evaluated according to the method described previously (Gwazdauskas et al., 1978). Retained fetal membrane was defined as a failure to release fetal membranes within 24 h of calving as described (Khorsandi et al., 2016). Body weight (BW) and body condition score (BCS) were recorded weekly. Body weight was measured using a cow weight tape scale after morning milking and body condition score (BCS) was evaluated and assigned to each cow using a quarter-point scale from 1 to 5 as described (Ferguson et al., 1994). Cows were milked twice daily and milk yields were recorded at each milking weekly, and milk composition (fat, protein, lactose and somatic cell counts) was determined weekly according to approved procedures of AOAC (1985). Somatic cell count was measured from individual milk samples during rainy season (May to September) and out of rainy season (October to April). Feed offered and rejected were recorded daily throughout the experimental period for each treatment group for dry matter intake (DMI; kg/day) calculation.

Ovaries of all cows were scanned daily using an ultrasound machine (Real-time, B-mode, 7.5 MHz transrectal transducer (linear array), HS-2000, Honda Electronics Co., Ltd., Japan), starting 7 days postpartum until first ovulation occurred or day 60 postpartum (if ovulation has not occurred). Ovulation was determined by the disappearance of the largest follicle followed by the formation of a CL on the same location. Day of first estrus was determined from day 7 after parturition until first estrus or day 60 postpartum (if estrus was not detected). All cows were observed twice daily for at least 30 min before milking. Ovulatory response, size of follicle/ preovulatory follicle, corpus luteum responsive of PGF2α, and ovulation rate after the first GnRH and second GnRH present on the ovaries of each cow were examined by transrectal ultrasonography during the modified Ovsynch protocol (days 60, 67, 69, and day 0 of TAI; Figure 1). Conception rate was determined on day 42 after TAI for all cows by transrectal ultrasonography described above (Figure 1).

Collection of follicular fluid

Only dairy cows with lactation number ≥ 4 were used for the collection of follicular fluid. Cows from each treatment were randomly assigned to be slaughtered on day 84 postpartum or 14 days after TAI (during diestrus phase of the estrous cycle; Figure 1) for collecting ovaries (control; n = 4 and OTM; n = 4). After slaughter, the ovaries of the cows were excised immediately and placed in ice-cold 0.05 M phosphate-buffered saline (PBS) and swiftly transported to the laboratory. For both ovaries from each cow, the number and diameter of all visible follicles were recorded. The follicular fluid was gently aspirated from mapped small (3-5 mm), medium (6-9 mm) and large (10-20 mm) follicles into 1.5 mL tubes as previously described (Moonmanee et al., 2013). Follicular fluid was centrifuged at 1000 x g for 10 minutes to remove cells and cumulus-oocyte complexes, and the supernatant was stored at -20 °C until analysis for concentrations of E2 and progesterone (P4).

Hormone assays

Blood samples (10 ml) from all cows were collected by puncture of the coccygeal veins during the modified Ovsynch protocol (as days 60, 67, 69, and 70 postpartum, respectively; Figure 1) and after TAI on days 5, 8, 12, 15, 18, 21 and 42 (as days 75, 78, 82, 85, 88, 91 and 112 postpartum, respectively; Figure 1) for quantification of serum P4 concentrations. Concentrations of serum P4 were evaluated by commercial ELISA kit (DRG Instruments, Marburg, Germany). The intra-assay coefficient of variation was 6.65% and sensitivities were 0.025 ng/mL for P4, as previously described (Navanukraw et al., 2015). Concentrations of P4 and E2 in unextracted follicular fluid (1:100 and 1:250 for P4 and E2) were determined by commercial ELISA kits (DRG Instruments, Marburg, Germany). Intra-assay coefficient of variation was 7.85% for P4 and 6.05% for E2, and assay sensitivities were 0.025 ng/mL for P4 and 10.0 pg/mL for E2, as described (Moonmanee et al., 2013).

Serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities

Blood samples were collected from each cow by puncture of the coccygeal veins at 21 days prior to the expected calving date (-21 days) and on days 21, 60, and 84 postpartum (Figure 1) for serum SOD and GSH-Px activities analysis. Serum SOD and GSH-Px activities were measured by Superoxide Dismutase Assay Kit and Glutathione Peroxidase Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan, USA), following the manufacturer's instructions, as described (Bicalho et al., 2014).

Statistical analysis

Data are presented as mean±SEM. Continuous data were analyzed using procedure GLM of SAS (SAS Institute Inc., Cary, NC), whereas conception rate was analyzed using Chi-square analysis and logistic regression. Repeated measurements on milk yields, DMI, follicular fluid E2 and P4 concentrations, serum P4 concentrations, and antioxidant enzymes were analyzed using PROC MIXED procedure (SAS Inst. Inc., Cary, NC), and differences between specific means were evaluated by least significant difference (Steel et al., 1997).

RESULTS

During prepartum period, DMI, BCS, and BW were similar for control and OTM cows. However, during postpartum period, cows fed OTM had greater BCS and BW, than the control group ($P < 0.05$; Table 2). Parturition score was less ($P < 0.05$) in cows fed OTM. Percentage of RFM was not different between the groups, whereas placental expulsion period in control cows was longer than cows fed OTM (13.2 and 6.3 hours; $P < 0.05$; Table 2). After parturition, DMI in control cows and OTM cows declined during first two weeks after parturition and increased later (Figure 2A). However, DMI was greater in OTM cows than control cows during 3-12 weeks postpartum ($P < 0.05$; Figure 2A).

Table 2 Effect of treatment on production parameters, parturition score, and health parameters of dairy cows

	Treatments	
	Control	OTM
Prepartum dairy cows		
Dry matter intake (kg/day)	12.5 ± 0.13	12.8 ± 0.16
Body condition score	3.1 ± 0.03	3.1 ± 0.03
Body weight (kg)	509.9 ± 2.15	507.2 ± 2.24
Postpartum dairy cows		
Body condition score	2.5 ± 0.04[b]	2.9 ± 0.04[a]
Body weight (kg)	430.9 ± 5.16[b]	443.3 ± 4.37[a]
Parturition score	1.7 ± 0.12[a]	1.2 ± 0.08[b]
Retained fetal membranes, % (no./no.)	16.7 (5/30)	3.3 (1/30)
Placental expulsion period (h)	13.2 ± 2.07[a]	6.3 ± 0.73[b]

[a,b] Within a row, means with different superscripts differ ($P < 0.05$)

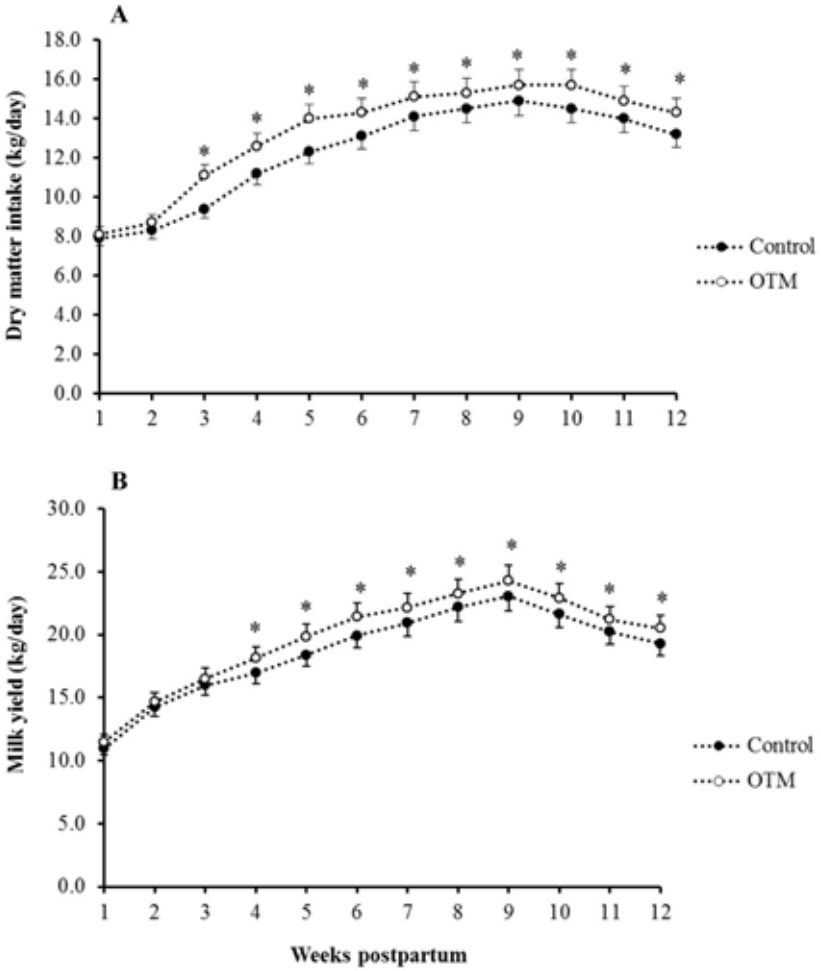

Figure 2 Dry matter intake (DMI; A) and milk yield (B) by week with two groups of cows fed OTM (white circle) and control (black circle). *Different from the control, $P < 0.05$.

Soon after parturition, milk yields were not different between groups. Nevertheless, milk yields were greater in OTM cows than control cows during 4-12 weeks postpartum ($P < 0.05$; Figure 2B). The interaction between treatments and days of milk yield was observed ($P < 0.01$). Average milk yield throughout the experiment was greater ($P < 0.05$; Table 3) for the OTM cows compared with control cows, whereas, milk compositions were similar between control and OTM groups. Somatic cell numbers of OTM cows during rainy season were lower than those of control cows ($P < 0.05$; Table 3). The mastitis problem was not occurred in control cows and OTM cows.

Table 3 Effect of treatment on milk performance of dairy cows

	Treatments	
	Control	OTM
Average milk yield (kg/day)	18.63 ± 0.13[b]	19.70 ± 0.12[a]
Milk composition, %		
Fat	3.42 ± 0.06	3.47 ± 0.05
Protein	3.10 ± 0.02	3.07 ± 0.02
Lactose	4.57 ± 0.02	4.61 ± 0.02
Solid not fat	8.46 ± 0.07	8.52 ± 0.05
Total solids	12.14 ± 0.11	12.21 ± 0.08
Somatic cell counts (x 10^3)		
Rainy season	396 ± 8.20[a]	365 ± 5.70[b]
Out of rainy season	288 ± 3.80	282 ± 4.90

[a,b] Within a row, means with different superscripts differ ($P < 0.05$)

Day to first ovulation (19.5 and 29.8 days; $P < 0.05$; Table 4) and day to first estrus (36.6 and 52.3 days; $P < 0.05$; Table 4) of cows fed OTM occurred sooner than those control cows.

In addition, cows fed OTM had a greater percentage of first estrus and size of preovulatory follicles than the control group ($P < 0.05$; Table 4). However, percentage of corpus luteum responsive of PGF2α, synchronized ovulation rates after first and second GnRH and conception rates did not differ between the groups (Table 4).

Table 4 Effect of treatment on reproductive performance parameters of dairy cows

	Treatments	
	Control	OTM
Day to first ovulation (day)	29.8 ± 0.6[a]	19.5 ± 0.3[b]
Day to first estrus (day)	52.3 ± 1.2[a]	36.6 ± 1.0[b]
First estrus, % (no./no.)	60.0 (18/30)[b]	93.3 (28/30)[a]
Size of dominant follicle at first GnRH (mm)	11.7 ± 0.3	11.9 ± 0.3
Size of preovulatory follicle (mm)	13.2 ± 0.1[b]	15.4 ± 0.2[a]
Corpus luteum responsive of PGF2α, % (no./no.)	90.0 (27/30)	93.3 (28/30)
Synchronized ovulation rate		
- After the first GnRH, % (no./no.)	86.7 (26/30)	90.0 (27/30)
- After the second GnRH, % (no./no.)	90.0 (27/30)	96.7 (29/30)
Conception rate, % (no./no.)	36.7 (11/30)	56.7 (17/30)

[a,b] Within a row, means with different superscripts differ ($P < 0.05$)

Before timed AI, mean serum P4 concentrations at the time of the first GnRH injection were greater for OTM cows than those control cows (1.86 vs. 1.09 ng/mL; $P < 0.05$; Figure 3). However, there were no differences in the mean serum P4 concentrations at the time of PGF2α and second GnRH injections or at the day of TAI (Figure 3). From days 0 to 8 after TAI, serum P4 concentrations did not differ between the groups. However, serum P4 concentrations in OTM cows were greater ($P < 0.05$; Figure 4) than control cows on days 12 (4.5 vs. 3.7 ng/mL), 15 (5.1 vs. 3.8 ng/mL), 18 (5.2 vs. 3.7 ng/mL), 21 (5.0 vs. 3.0 ng/mL), and 42 (5.6 vs. 3.6 ng/mL).

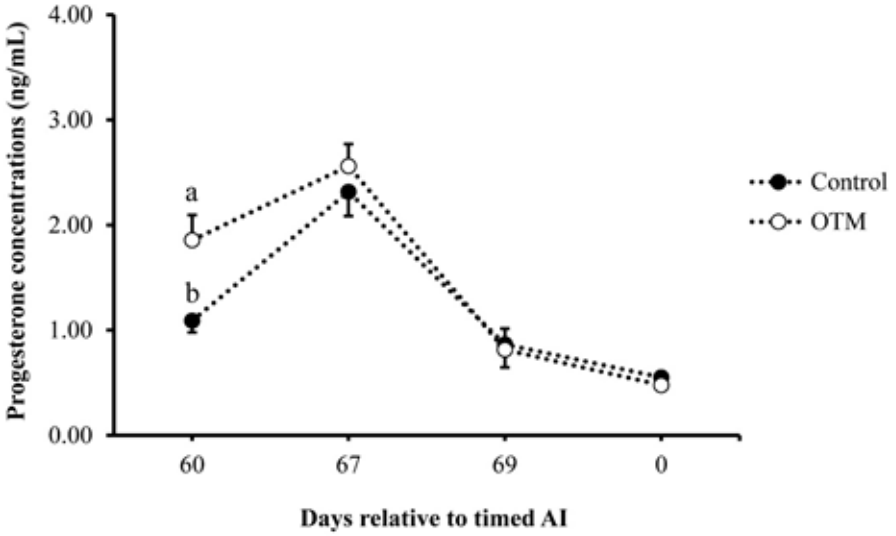

Figure 3 Effect of treatment on serum progesterone concentrations (ng/mL) on days relative to timed AI. a,bValues are significantly different ($P < 0.05$).

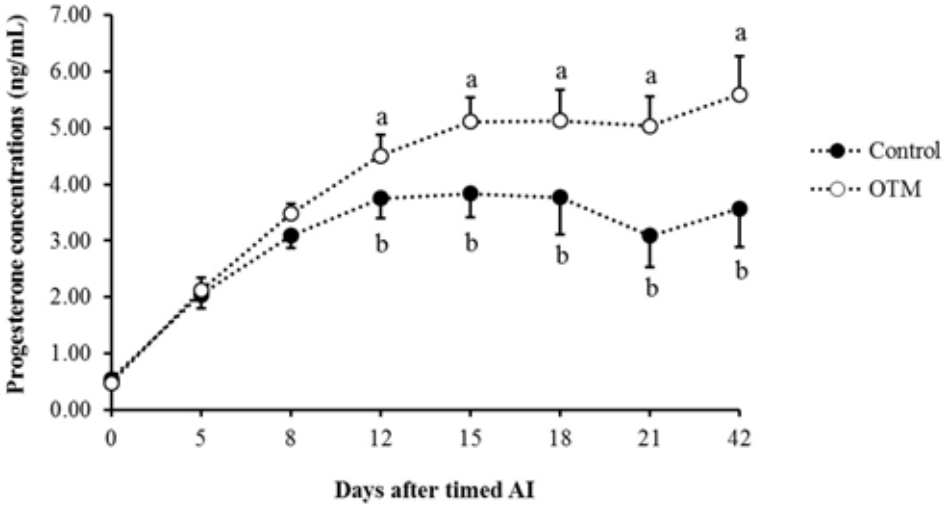

Figure 4 Effect of treatment on serum progesterone concentrations (ng/mL) after timed AI. [a,b]Values are significantly different ($P < 0.05$).

Cows fed the OTM had greater number of small and large follicles as well as size of large follicle ($P < 0.05$; Table 5) than the control cows. Concentrations of P4 and E2/P4 ratio in follicular fluid did not differ in small, medium and large follicles between control and OTM groups. However, large follicles of cows fed with OTM have greater concentrations of E2 in follicular fluid when compared with the control cows (376.9 and 336.3 ng/mL; $P < 0.05$; Table 5), which indicated that the large follicles of cows fed with OTM had a greater ability to produce E2.

Table 5 Effect of treatment on follicle number, follicle size, follicular fluid E2 and P4 concentrations, and E2/P4 ratio of the small (3-5 mm), medium (6-9 mm), and large (10-20 mm) follicles in dairy cows

	Treatments	
	Control	OTM
Follicle number		
Small follicle	10.3 ± 0.5^{b}	12.2 ± 0.7^{a}
Medium follicle	3.7 ± 0.6	4.1 ± 0.5
Large follicle	1.6 ± 0.3^{b}	2.5 ± 0.2^{a}
Follicle size		
Small follicle	4.5 ± 0.3	5.1 ± 0.3
Medium follicle	7.8 ± 0.2	8.0 ± 0.3
Large follicle	11.1 ± 0.1^{b}	12.6 ± 0.4^{a}
E2 (ng/ml)		
Small follicle	117.6 ± 4.7	125.2 ± 7.0
Medium follicle	273.9 ± 9.4	278.0 ± 8.7
Large	336.3 ± 9.9^{b}	376.9 ± 9.6^{a}
P4 (ng/ml)		
Small follicle	46.4 ± 6.2	47.6 ± 7.0
Medium follicle	67.6 ± 7.7	73.4 ± 6.3
Large follicle	81.2 ± 8.7	86.3 ± 7.2
E2/P4 ratio		
Small follicle	2.62 ± 0.3	2.56 ± 0.1
Medium follicle	3.83 ± 0.3	3.75 ± 0.4
Large follicle	4.21 ± 0.3	4.23 ± 0.4

[a,b] Within a row, means with different superscripts differ ($P < 0.05$)

Serum SOD activity in the OTM cows were greater ($P < 0.01$; Figure 5A) than the control cows on days 21 (21.1 vs. 10.5 U/mL), 60 (17.5 vs. 11.0 U/mL) and 84 (12.5 vs. 9.5 U/mL). Serum GSH-Px activity in the OTM cows were greater ($P < 0.01$; Figure 5B) than the control cows on days 21 (16.1 vs. 11.2 U/mL), and 60 (14.2 vs. 10.4 U/mL). In addition, concentrations of mean serum SOD and GSH-Px in the cows fed OTM were greater ($P < 0.05$) than those control cows (15.5 vs. 10.6 U/mL and 12.1 vs. 9.9 U/mL). On-farm growth performance of the chickens at different week intervals in the three agro-ecologies are reported in Table 5. Genotypes and sex had highly significantly ($p<0.0001$) influenced the growth performance of the chickens within each agroecology. In all cases Sasso breed showed significantly ($p<0.0001$) higher body weight in all weeks of their age at each agroecology. Male chickens were highly significantly heavier than the females in all agro-ecologies.

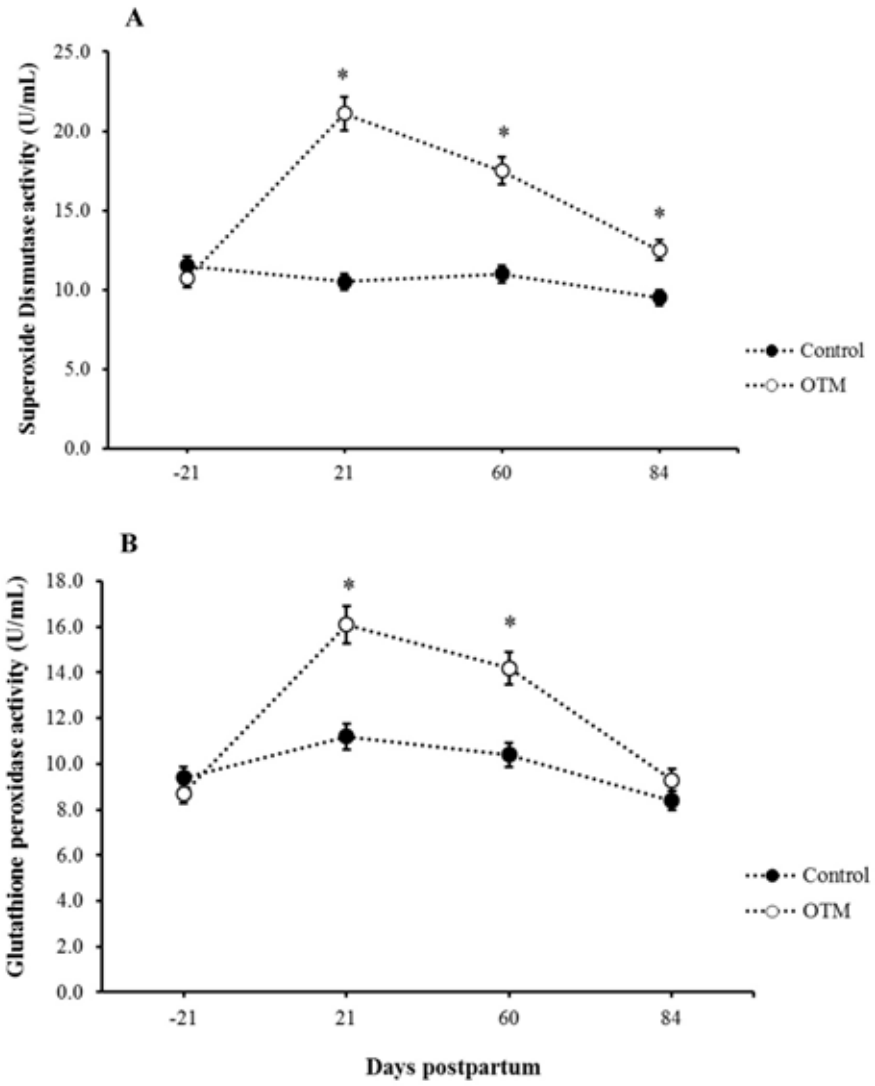

Figure 5 Effect of treatment on serum superoxide dismutase (SOD; A) and glutathione peroxidase (GSH-Px; B) concentrations (U/mL); the white circle illustrates the average SOD and GSH-Px activity for cows fed OTM and black circle illustrated the SOD and GSH-Px activity for the control cows. *Different from the control, $P < 0.01$

DISCUSSION

The OTM supplement affects several physiological processes which compromise cell growth, metabolic, immune and reproductive functions. The present study demonstrated that cows fed OTM during pre- and postpartum resulted in greater BCS, BW, parturition score, placental expulsion period, DMI, milk yield, antioxidant enzymes (SOD and GSH-Px), and reproductive performance parameters. Several previous studies reported a positive effect of OTM on milk yield (Nocek et al., 2006; Griffiths et al., 2007; Siciliano-Jones et al., 2008). However, our study indicated that milk compositions did not differ between control and OTM groups ($P > 0.05$). Nevertheless, somatic cell counts of OTM cows during rainy season were lower than those of control cows ($P < 0.05$). Similarly, a recent study reported a lower incidence of mastitis and

decreased somatic cell counts were observed in cows supplement with trace mineral solution containing Zn, Cu, Mn, and Se (Machado et al., 2013). Cows supplemented with OTM increased milk yield; however, most of the increase in milk yield was driven by greater feed intake in cows offered OTM (Cope et al., 2009).

Retained fetal membrane has defined as the failure to expel the fetal membranes within 12-24 h after calving (Drillich et al., 2003). Risk factors associated with the RFM are including induced parturition, length of gestation, abortion, twinning, dystocia, imbalances in nutrition such as vitamin E and Se, and immunosuppression (Beagley et al., 2010). Negative consequences to the RFM include delayed uterine involution, increased postpartum disease, and ovarian functions such as slow growth of first postpartum dominant follicle, decreased P4 and E2 productions, and increased days open (Stevens and Dinsmore, 1997; Sheldon et al., 2009; Beagley et al., 2010; Bicalho et al., 2014). Lower prepartum activities of placental SOD, GSH-Px, and E2 concentrations were found in cows that subsequently developed the RFM (Wischral et al., 2001). In the present study, percentage of RFM was not different between the groups, whereas placental expulsion period in control cows was longer than cows fed OTM. These results indicated that SOD and GSH-Px may play a role in uterine and placental health being part of the antioxidant defense system (Sordillo and Aitken, 2009). Cows with uterine inflammation and poor placental health have negative effects on reproductive performance, which suppress follicular growth and E2 production, and LH pulse secretion (Sheldon et al., 2009; Cheong et al., 2017). Concentration of follicular fluid E2 of large follicles was greater in cows fed OTM than that of those control cows indicating that OTM affects the resumption of ovarian function in postpartum cows. The normal pattern of early resumption of ovulation may be delayed in high-yielding Holstein-type cows normally because of the effects of dystocia, RFM, and uterine infections. Therefore, OTM may contribute to the mechanism of immune function and oxidative metabolism particularly during the transition period (Overton and Yasui, 2014).

Trace mineral supplement has been shown to improve fertility in cattle (Griffiths et al., 2007; Rabiee et al., 2010). Our experiment demonstrated that OTM cows had a positive effect on day to first ovulation and estrus, percentage of cows exhibiting estrous behavior, and size of preovulatory follicle. Several studies have suggested that the OTM improved reproductive performance including a decrease in days to first estrus after parturition (Campbell et al., 1999), and an increased in the percentage of cows pregnant at 150 DIM (Nocek et al., 2006). Other studies have also reported that Zn and Cu play important roles in reproductive function of dairy cows, reduced days to first service, lower services per conception, and decreased days open (Kappel et al., 1984). Especially, dairy cows supplemented with OTM during late gestation or 3 weeks after calving have beneficial effects on fertility (Campbell and Miller, 1998). In this study, corpus luteum responsive to PGF2α and synchronized ovulation rates after first and second GnRH were higher in control and OTM cows, but the conception rate was 36.7% and 56.7%, respectively. Similarly, several authors using a TAI protocol have also reported a lower conception rate (Pursley et al., 1997; Navanukraw et al., 2004). In this regard, the size of follicles after the second GnRH of the Ovsynch protocol may not optimal-sized

follicles, which result in increased embryonic and pregnancy loss in dairy cows (Colazo and Ambrose, 2012). Moreover, dairy cows in tropical countries (Thailand) are associated with increased oxidative stress that influences ovarian function, oocyte health, uterine environment, and embryonic development (Hansen, 2007; Navanukraw et al., 2015).

Serum P4 concentrations in OTM cows were greater than control at the time of first GnRH injection and on days 12, 15, 18, 21, and 42 after TAI. It is possible that supplementation of OTM may affect serum P4 concentrations, because Mn is necessary for cholesterol synthesis, which, in turn, is required for synthesis of the E2 and P4 (Siciliano-Jones et al., 2008). Elevated serum P4 during preovulatory follicle development is associated with greater fertility in cows submitted to AI after natural estrus, after PGF2α-induced luteolysis or during TAI protocols (Martins et al., 2011; Pereira et al., 2017). Moreover, in our study, cows fed the OTM had a greater number of small and large follicles as well as size of large follicles. Greater P4 concentrations have a positive effect on follicular turnover increasing the number of young large follicles with the potential to ovulate and subluteal concentrations extend the growth of follicles (Navanukraw et al., 2014; Thammasiri et al., 2016).

Our experiment clearly demonstrated that the follicular fluid concentrations of E2 in large follicles in OTM cows were greater than control cows. Estradiol is recognized as the follicular growth, differentiation, and survival factor (Moonmanee et al., 2013). A previous study suggested that intake of OTM, especially Se affects ovarian steroid biosynthesis such as E2 and P4 (Cerny et al., 2016). Thus, OTM may provide a suitable approach to enhance fertility in dairy cows by increasing size of follicle and steroid hormone (E2 and P4) concentrations. However, the ratio of E2/P4 in follicular fluid samples did not differ in small, medium, and large follicles between control and OTM groups.

Greater serum SOD and GSH-Px activities were significantly observed during 21-60 days in milk in cows fed OTM supplement. These observations were in agreement with a recent study Machado et al. (2014) in which trace mineral supplementation in cows increased serum SOD activity. A previous study reported that trace mineral supplements increased serum neutrophil function and GSH-Px activity, which was attributed to improved repair of damaged uterine tissue following calving (Ahola et al., 2004). The higher reactive oxygen species production than antioxidant defense mechanism may lead to oxidative stress and cause tissue damage (such as metritis). Greater bioavailability of the OTM may contribute to increased cellular protection by the proliferating cells from ROS produced via their metabolism, through the increased enzymatic activity (Teixeira et al., 2014). Selenium is an essential component of GSH-Px enzymes destroying hydrogen peroxide and lipid hydroperoxides (Rotruck et al., 1973). The Cu-Zn superoxide SOD plays a key role in dismutation of superoxide radicals to hydrogen peroxide in the cytoplasm (Halliwell, 1999). Regarding the particular immune cell sensitivity to oxidative stress, the function of the OTM may support the enhanced antioxidant status (Teixeira et al., 2014). The OTM supplementation appears to affect concentrations of antioxidant enzymes, health, steroid hormones, and reproductive performance in dairy cows through a role in the mechanism of immune function, oxidative, and steroid metabolism (Bicalho et al., 2014; Overton and Yasui, 2014).

CONCLUSIONS

In conclusion, although the interactions among organic trace minerals, fertility, utero-placental and ovarian function and immune function are substantially complex, OTM supplements for transition dairy cows with synchronization of ovulation and TAI protocol enhance uterine and placental health, milk yield, SOD and GSH-Px activities, and concentrations of E2 and P4. Future studies are needed to elucidate specific mechanisms by which the OTM enhances steroid metabolism, immune response, thus uterine health of postpartum dairy cows.

AUTHOR CONTRIBUTIONS

Vilaivan Khanthusaeng: conducting the experiment, collection of samples, validation and optimization of hormonal and enzymes analyses, and preparing the original draft; Thanya Bunma: hormonal administration, animal handling, and data acquisition; Chutikun Kanjanaruch: data acquisition and manuscript review; Jiratti Thammasiri: analyzed the data and editing the manuscript; Chainarong Navanukraw: supervision, study concept and experimental design, data interpretation and preparing and editing the manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest in this study.

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