

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.809.328>

Effect of Peripartum Organic Zinc and Copper Supplementation on Blood Metabolic and Hormonal Profiling of Primiparous Buffaloes

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ABSTRACT

This study evaluated effects of organic trace mineral supplementation on hormonal and metabolic profile of primiparous buffaloes. Fourteen animals were randomly divided into two groups control (n=7) and treatment (n=7; Copper@225mg, Zinc@1.0gm per animal per day). Treatment group was supplemented with organic copper at the rate of 225mg and Zinc at rate 1.0gm per animal per day respectively, in addition to normal feeding, 60 days before the expected date of calving till the date of artificial insemination. A significant (p<0.05) decrease in the serum non-esterified fatty acid (NEFA) levels ($\mu\text{mol/l}$) was observed in supplemented (388.88 ± 3.73 to 334.40 ± 2.86 $\mu\text{mol/l}$) than control group (405.27 ± 2.63 to 352.92 ± 5.45 $\mu\text{mol/l}$) buffaloes. There was significant difference (p<0.05) in total protein (g/dl) values between treatment (6.90 ± 0.06 to 7.35 ± 0.05 g/dl) and control group (6.22 ± 0.20 to 6.35 ± 0.22 g/dl). Total protein (g/dl) increased after parturition in treatment group, while in control group the concentration of total protein did not change significantly after parturition. No significant difference was observed in progesterone (0.33 ± 0.01 to 1.83 ± 0.06 ng/ml vs. 0.26 ± 0.01 to 1.29 ± 0.08 ng/ml) and estradiol (22.67 ± 0.42 to 140.82 ± 0.51 pg/ml vs. 21.04 ± 0.16 to 145.23 ± 0.39 pg/ml) concentration in supplemented and control group, respectively. We concluded that supplementation of organic trace mineral significantly improved the total protein and non-esterified fatty acid levels in blood thus helps in proper mobilisation and utilisation of body reserves but it did not affect the blood hormonal profile significantly.

Keywords

Trace Minerals,
Buffalo, Metabolic
profile, Hormonal
Levels

Article Info

Accepted:

24 August 2019

Available Online:

10 September 2019

Introduction

World buffalo population is approximately 170 million with 97 percent in Asia (FAO

2004). India is possessing 56 percent of world buffalo population. The production potential of buffaloes is constrained by its low reproductive efficiency due to higher age of

puberty, poor conception rates, longer service period and calving interval. The success of the dairy buffalo economy lies in optimal reproductive cycle of each individual buffalo in the herd within normal physiological range (Dhaliwal 2005). Any deviation in the breeding cycle results in a progressive economic loss due to prolonged dry period and reduced calvings and lactations during the life span of the animal (Singh *et al.*, 2006). Minerals have basic key role in maintenance of metabolism and studies on their nutritional requirements in the body led to the classification of these minerals as macro and micro minerals. Adequate mineral intake and absorption is required for a variety of metabolic functions including immune response to pathogenic challenge, reproduction and growth (Garg *et al.*, 2009).

Copper is mainly stored in the liver and is an enzyme component of essential metabolic processes. As an enzyme activator, it provides strong bones and joints and is heavily involved in the utilization of iron and thus the synthesis of hemoglobin. Through the promotion of superoxide dismutase, which deactivates free oxygen radicals, copper is also involved in cell protection and healthy immune system. Copper as a component of enzymes like peptidylglycine α -amidating monooxygenase (PAM) and dopamine β -monooxygenase plays an important role in the activation of gonadotropin releasing enzyme (Michaluk and Kochman 2007). Cu appeared to be the cause of delayed puberty (possibly due to depressed basal LH release, affecting follicular estradiol production), reduced conception rate, and failure to ovulate (Phillippo *et al.*, 1987). Copper interact with granulosa cells for production of estrogen. Altered plasmatic level of Cu confers changes in pattern and level of steroidal hormone synthesis leading to decline in overall fertility and altered reproductive behaviour in females eg. nymphomania in ewe (Hidioglou 1979).

Zinc is the second most common trace element. It acts as an activator and a component of more than 300 enzymes and hormones. Zinc plays an important role in metabolism, protein biosynthesis and the regulation of gene activity. Due to its crucial function in defense enzymes (copper-zinc superoxide dismutase), zinc deficiency reduces resistance. In dairy animals zinc deficiency signs can be seen as bad hoof and horn quality, reduced fertility and poor udder health. Zinc is considered to be vital for proper sexual maturity, reproductive efficiency, regulation and onset of estrus (Green *et al.*, 1998). GnRH secretion in the body is regulated by plasma zinc levels which is essential for secretion and maintaining the activity of FSH and LH (Das *et al.*, 2009).

During periods of zinc deficiency due to alterations in synthesis and secretion of these hormones leads to arrest of ovulation, erratic estrus cycles and abnormal reproductive performance of animal (Kaswan and Bedwal 1995). Zinc takes part in maintenance of epithelial integrity of uterine lining in cattle for implantation of embryos and its insufficient levels is found to be associated with abortion, fetal mummification, lower birth weights and prolonged labor (Kumar *et al.*, 2011). Stanton *et al.*, (2000) reported that cows receiving organic trace minerals exhibited higher pregnancy rates to AI than those receiving inorganic trace minerals. Bisla *et al.*, (2006) and Kumar (2008) recorded more number of animals exhibited estrus and improved in the conception rate in postpartum anoestrus buffaloes with mineral supplementation. Jyoti Sharma *et al.*, (2009) observed estrus in 66.67 per cent of the cattle fed with concentrate feed containing dicalcium phosphate, copper sulphate and magnesium sulphate. Transition period is an important and vulnerable period encountered by dairy cow that extends from three weeks before and after calving (Curtis *et al.*, 1985,

Grummer 1995). Dairy cow experience about one-third decrease in feed intake during the last three weeks prior to calving, with significant reduction observed in the final week before parturition (Hayirli *et al.*, 2002). This is mainly due to increase in concentration of circulating estrogen and less capacity for rumen to expand because of increased foetus size. After calving, the cow which was already consuming low proportion of dietary energy, mobilizes fat (NEFA) from adipose tissues as a source of energy for maintenance of body functions and to support milk production resulting into negative energy balance (Moore *et al.*, 2005). Circulating concentration of blood metabolites like NEFA and BHBA have negative effect on post-partum fertility leading to anoestrous, low conception rates, long calving interval with decreased survivability of embryo in subsequent pregnancies (Staples *et al.*, 1990).

Materials and Methods

Location

All procedures were approved by the Institutional Animal Ethics Committee (IAEC: GADVASU/2018/IAEC/45/01). Study was conducted on 14 primiparous buffaloes being reared at Directorate Livestock Farm, Guru Angad Dev Veterinary and Animal Sciences University, Punjab, India (30.9°N, 75.85°E and 256 m above sea level), where the climate is humid sub-tropical with defined seasons.

Selection of Animals

All the buffaloes selected were in first parity having a body condition score of ≥ 3 . Buffaloes were selected 60 days before calving and maintained until artificial insemination. All these buffaloes were maintained under general managerial practices as followed for pregnant animals in the herd at the Directorate Livestock Farm,

GADVASU, Ludhiana. The feed and water were available ad lib to these animals. At the beginning of the experiment the average age and body weight of buffaloes in control group was 783.75 ± 40.51 days and 548.33 ± 32.91 kg and in treatment group was 716.67 ± 42.82 days and 562.0 ± 38.65 kg. Animals were housed in semi conventional housing system during the months of January 2018 to May 2019. Animals were divided into two groups control (n=7) and treatment (n=7).

Feeding

The nutrient requirements of the animals were mostly met with ad lib green fodder and measured amount of concentrate. The green fodders grown in the institute farm, were supplied according to the seasonal availability. The concentrate was fed at the rate of 2.5 kg/day per animal for body maintenance to heifers. For pregnant animals 1kg/day/animal (upto seven months) and 2 kg/day/animal during advanced pregnancy (last 90 days). Milking buffaloes were given additional concentrate at the rate of 1.0 kg for every 1.5 kg milk production, above 5.0 kg milk yield. The concentrate to the milking animals was fed in divided allowances during milking. The diets provided for pre and post-calving cows, as well as the chemical analyses are shown in Table 1 and 2. Control group (n=7) was fed as per the standards followed at Dairy Farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Treatment group (n=7) was supplemented with organic copper and zinc supplementation in addition to normal feeding, Copper@225mg, Zinc@1.0gm per animal per day, 60 days before the expected date of calving till the date of artificial insemination.

Blood Sampling

All buffaloes were subjected to blood sampling (10 ml), through jugular vein-

puncture at weekly interval after parturition until artificial insemination in both group I and II. Blood samples were collected into spinwin conical tubes (Tarsons Products Pvt. Ltd. Maharashtra, India) which were immediately placed in the icebox and transferred to the laboratory. Serum was harvested by centrifugation (3000 rpm, 15 minutes) and stored at -20°C until the hormonal and metabolic profiling.

Hormonal and metabolic profiling

Serum progesterone and estradiol estimation was done using a solid phase enzyme immunoassay kits. ELISA kits used for P₄ and E₂ were manufactured by XEMA Co. Ltd (Moscow, Russia) with catalog number K207 and K208 respectively. The sensitivity of the progesterone and estradiol assay was 0.6 ng/ml and 62.4 pg/ml.

For estimation of serum NEFA sandwich ELISA KIT manufactured by Bioassay Technology Laboratory (Shanghai, China) with catalog number E0021Bo was used. Total protein estimation was done using the VITROS chemistry products TP Slides (Ortho Clinical Diagnostics, Mumbai, India) and the VITROS Chemistry Products DT Calibrator Kit on VITROS DT 60/DT60 2 Chemistry Systems (Ortho- Clinical Diagnostics, Johnson and Johnson, SA).

Statistical analyses

Data generated by ultrasound examination and hormone assay were subjected to statistical analysis using IBM SPSS Statistical Version 23 (SPSS 23.0 for windows; SPSS, Chicago, IL, USA). To compare the effect of treatment on serum P₄ concentration (ng/ml), serum E₂ concentration (pg/ml), TP value (g/dl) and NEFA concentration (µmol/l) over days within the group, data were analyzed using “One way ANOVA”. The whole of the analyzed data is

presented as Mean ± SEM. A probability level of (p < 0.05) was considered significant.

Results and Discussion

Blood metabolic profile in postpartum primiparous buffaloes fed trace mineral supplement

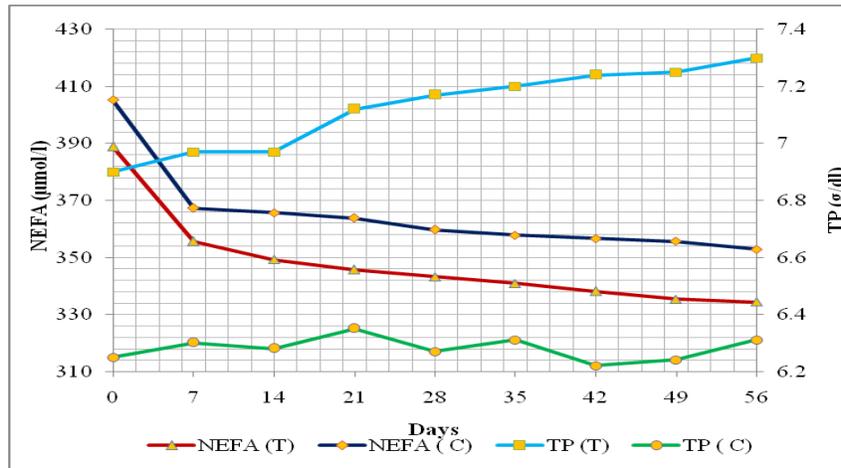
Mean serum NEFA values obtained across different days in treatment and control group are presented in Table 3. Concentration of NEFA were highest at the day of calving (388.88±3.73 and 405.27±2.63 µmol/l) and lowest on day 56 postpartum (334.40±2.86 and 352.92±5.45 µmol/l) in treatment and control groups respectively. A significant (p<0.05) decrease in the serum NEFA levels (µmol/l) was observed in supplemented than control group buffaloes.

Concentration of NEFA peaked on the day of calving and then decreased on day 7 postpartum in both the groups but decrease was more rapid in treatment group as compared to control group (Figure 1), and is in affirmation of studies by Vazquez-Anon M *et al.*, (1994) and Grum DE *et al.*, (1996). Accorsi *et al.*, reported higher NEFA levels until first 10 days after calving. Yang WZ *et al.*, (1996) reported that control buffaloes had higher NEFA concentrations when compared to buffalo receiving chromium, suggesting greater mobilization of body reserves. Nonesterified fatty acids and BHBA are considered as markers of negative energy balance during the transition period (Ospina *et al.*, 2010 a,b, Chapinal *et al.*, 2011, McArt *et al.*, 2012 b). Elevation of NEFA or BHBA concentrations during pre- and postpartum periods have been associated with negative downstream outcomes in individual animals, such as decreased milk production (Duffield *et al.*, 2009, Ospina *et al.*, 2010b, McArt *et al.*, 2012b), decreased reproductive function (Ospina *et al.*, 2010b, McArt *et al.*, 2012b,

Garverick *et al.*, 2013), increased risk of health disorders (LeBlanc *et al.*, 2005, McArt *et al.*, 2012a), and increased risk of removal from the herd (Ospina *et al.*, 2010a, Roberts *et al.*, 2012, McArt *et al.*, 2012b), as well as on a herd level basis (Ospina *et al.*, 2010c). Findings are suggestive of better feed

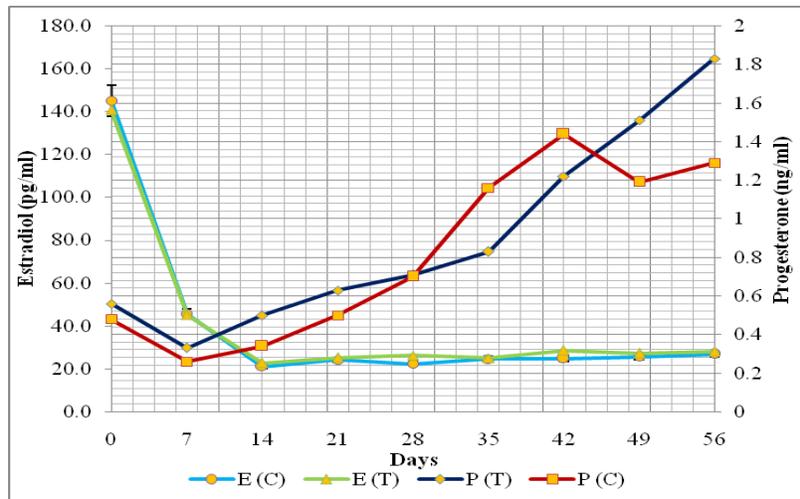
utilization and assimilation of available energy in supplemented group, treated animals maintained better energy balance and reproductive performance than the control animals. Serum total protein levels across different days in treatment and control groups are presented in Table 3.

Fig.1 Serum NEFA ($\mu\text{mol/l}$) and total protein (g/dl) comparison between treatment and control groups



NEFA (T): NEFA in treatment group; NEFA (C): NEFA in control group; TP (T): Total protein in control group; TP (C) Total protein in treatment group

Fig.2 Serum progesterone (ng/ml) and estradiol (pg/ml) comparison between treatment and control groups



E(C): Estradiol in control group; E (T): Estradiol in treatment group; P (T): Progesterone in control group; P (C) Progesterone in treatment group

Table.1 Ingredient composition of concentrate mixtures (%)

Ingredient	Maize	Soybean meal	Mustard cake	Full fat soya	Guar Korma	Cotton seed meal	Deoiled Rice bran	Mineral mixture	Calcite powder	Salt	Yeasac	Toxin binder	Vitamin mix
Concentrate Mixture (%)	40.00	10.00	20.00	2.00	3.00	4.00	16.7	2.00	0.50	1.50	0.10	0.10	0.10

Table.2 Composition of Mineral mixtures (%)

Type of mineral source	Di Calcium Phosphate	Limestone Powder	Magnesium Oxide	Magnesium Sulphate	Copper Sulphate	Iron Sulphate	Manganese Sulphate	Potassium Iodate	Cobalt Sulphate	Zinc Sulphate
Mineral Mixture (%)	59.77	21.98	07.83	04.98	0.427	01.50	0.427	0.050	0.050	02.99

Table.3 Blood NEFA and TP levels (Mean ± SEM) in postpartum primiparous buffaloes fed trace mineral supplement

Days	*NEFA (µmol/l)		*TP (g/dl)	
	Treatment (n=7)	Control (n=7)	Treatment (n=7)	Control (n=7)
0	388.88 ± 3.73	405.27 ± 2.63	6.90 ± 0.06	6.25 ± 0.23
7	355.61 ± 3.97	367.34 ± 2.29	6.97 ± 0.04	6.30 ± 0.21
14	349.27 ± 4.11	365.63 ± 3.14	6.97 ± 0.05	6.28 ± 0.15
21	345.65 ± 4.35	363.82 ± 2.60	7.12 ± 0.05	6.35 ± 0.22
28	343.24 ± 4.30	359.70 ± 3.88	7.17 ± 0.05	6.27 ± 0.20
35	340.93 ± 4.38	357.86 ± 4.89	7.20 ± 0.11	6.31 ± 0.17
42	338.07 ± 3.56	356.59 ± 4.85	7.24 ± 0.11	6.22 ± 0.20
49	335.35 ± 3.49	355.70 ± 6.13	7.25 ± 0.07	6.24 ± 0.14
56	334.40 ± 2.86	352.92 ± 5.45	7.35 ± 0.05	6.31 ± 0.16

*Significant difference at p<0.05 level

Table.4 Blood progesterone and estradiol levels (Mean ± SEM) in postpartum primiparous buffaloes fed trace mineral supplement

Days	Progesterone (ng/ml)		Estradiol (pg/ml)	
	Treatment (n=7)	Control (n=7)	Treatment (n=7)	Control (n=7)
0	0.56 ± 0.009	0.48 ± 0.02	140.82 ± 0.51	145.23 ± 0.39
7	0.33 ± 0.01	0.26 ± 0.01	45.17 ± 0.30	45.70 ± 0.17
14	0.50 ± 0.07	0.34 ± 0.01	22.67 ± 0.42	21.04 ± 0.16
21	0.63 ± 0.01	0.50 ± 0.006	25.25 ± 0.27	24.10 ± 0.40
28	0.71 ± 0.009	0.70 ± 0.01	26.32 ± 0.42	22.39 ± 0.46
35	0.83 ± 0.02	1.16 ± 0.07	24.98 ± 0.28	24.55 ± 0.27
42	1.22 ± 0.08	1.44 ± 0.04	28.52 ± 0.33	24.77 ± 0.19
49	1.51 ± 0.04	1.19 ± 0.02	27.33 ± 0.33	25.66 ± 0.27
56	1.83 ± 0.06	1.29 ± 0.08	28.16 ± 0.34	26.86 ± 0.47

Total protein in treatment group was highest (7.35±0.05 g/dl) and lowest (6.90±0.06 g/dl) on day 56 and 0 postpartum, respectively. While in control group the concentration was highest (6.35±0.22 g/dl) and lowest (6.22±0.20 g/dl) on day 21 and 42 postpartum, respectively.

Total protein increased after parturition in treatment group, while in control group the concentration of total protein did not change significantly after parturition. There was significant difference (p<0.05) in total protein

values between treatment and control group (Figure 1).

Results obtained were in affirmation with Nagalakshmi *et al.*, (2016), wherein, increased concentration of total protein was observed in animals supplemented with organic Zn compared to inorganic Zn. Shakweer *et al.*, (2010) also observed increase in total protein concentration due to zinc supplementation. Mousa and EL-Sheikh (2004) revealed that addition of 80 and 120 mg zinc sulfate improved total protein in

blood serum of lactating buffaloes. Similar findings, by Shakweer *et al.*, (2005), Shakweer and EL-Nahas (2005) and Shakweer *et al.*, (2006) found increased concentration of total protein with different level of zinc methionine supplementation.

As the calving approaches, negative energy balance and catabolism of body tissue increases which leads to degradation of the body fat and the body protein. DMI decrease is a prelude to decreased protein availability in the body leading to blood protein level reduction as ammonia is not available for the synthesis of amino acid. Strang *et al.*, (1998) reported that triglyceride loaded hepatocytes were less sensitive to the hormonal stimulation for albumin and protein synthesis than normal hepatocytes. Increased level of NEFA in the control group may be the reason of decreased level of total protein in the control group.

Hormonal levels in postpartum primiparous buffaloes fed trace mineral supplement

Serum progesterone values obtained across different days in the treatment and control groups are presented in Table 4. Higher progesterone concentration was 1.83 ± 0.06 ng/ml on day 56 and 0.33 ± 0.01 ng/ml on day 7 postpartum in the treatment group. In control group animals higher concentration was 1.44 ± 0.04 and 0.26 ± 0.01 ng/ml on day 42 and 7 postpartum, respectively. Progesterone concentrations were at baseline level within 24 hours from calving with a rising trend from day 7 postpartum in both treatment and control groups. Trace mineral supplemented buffaloes had slightly higher levels on all days except on day 35 and 42 postpartum wherein the control group had slightly higher values of progesterone (Figure 2). Serum progesterone values in this study corroborated well with the earlier reports in

buffaloes (Prakash and Madan 1986). Similar results were observed by Singh *et al.*, (2012). Dhama *et al.*, (2015) reported similar results in cattle. Kalasariya *et al.*, (2017) observed that mineral supplementation had no significant effect on progesterone concentration and was true for present work.

Mean serum estradiol values obtained across different days of the study in treatment and control groups are presented in Table 4. Estradiol was highest 140.82 ± 0.51 pg/ml on day of calving and lowest 22.67 ± 0.42 pg/ml on day 14 postpartum in treatment group while in control group the concentration was highest 145.23 ± 0.39 pg/ml and lowest 21.04 ± 0.16 pg/ml on day 0 and 14 postpartum, respectively. Estradiol values peaked on the day of parturition and showed a decreasing trend thereafter in both treatment and control group as depicted in Figure 4. Estradiol showed a rising trend from day 7 postpartum in both the groups.

Trace mineral supplemented group had slightly higher concentration of Estradiol on all days except on day 0 and 7 postpartum wherein control group had slightly higher values of Estradiol (Figure 2). Values obtained were similar to the findings of Kalasariya *et al.*, (2017) and also agreed to the trend of postpartum Estradiol profile reported by Singh *et al.*, (2012). Arya and Madan (2001) reported similar Estradiol values postpartum. Dhama *et al.*, (2015) observed similar results in cattle.

Supplementation of copper at the rate of 225mg and zinc at the rate of 1.0gm per animal per day significantly improves the total protein and non-esterified fatty acid levels in blood thus helps in proper mobilisation and utilisation of body reserves. However it did not affect the blood hormonal profile significantly but still there exist some numerical difference among their values from control group. Hence, chelated mineral

supplementation during pre and post-partum period is better option to improve the reproductive performance in primiparous buffaloes.

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How to cite this article:

Arashdeep Kaur, Prahlad Singh, Mrigank Honparkhe, R. S. Grewal, Ajeet Kumar, Ashwani Kumar Singh, Navdeep Singh, Shahbaz Singh Dhindsa and Ankit Kumar Ahuja. 2019. Effect of Peripartum Organic Zinc and Copper Supplementation on Blood Metabolic and Hormonal Profiling of Primiparous Buffaloes. *Int.J.Curr.Microbiol.App.Sci.* 8(09): 2858-2868.
doi: <https://doi.org/10.20546/ijcmas.2019.809.328>