EFFECT OF DIETARY ZINC SUPPLEMENTATION ON OVARIAN FOLLICULOGENESIS IN BUFFALO HEIFERS

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ABSTRACT

A study was undertaken to investigate the effect of zinc (Zn) supplementation on ovarian folliculogenesis in buffalo heifers. Eighteen buffalo heifers with an average body weight of 278.7±16.82 kg were randomly allotted to 3 groups in a completely randomized design. The dietary treatments were *viz.,* Basal diet (BD; 29.72 ppm Zn from feed ingredients), BD supplemented with 80 and 140 ppm Zn as $ZnSO_4$ $7H_2O$ (22% Zn). Heifers were fed the respective diets at 3% of body weight to meet the nutrient requirements (NRC, 2001) of dairy cattle except Zn throughout for an experimental period of 150 days. After 2 months of feeding trial, ovarian folliculogenesis study was made on all animals daily with help of B-mode linear array ultrasound scanner equipped with 7.5 MHz transducer. The proportion of large follicles (>8 mm) was higher (P<0.05) with 140 ppm (5.29%) Zn supplementation followed by 80 ppm (3.38%) compared to no Zn supplementation. However, no large sized follicles on ovary were observed in heifers supplemented with no Zn. While the mean number of small (3 to 5 mm) and medium follicles (5 to 8 mm) were not affected by Zn concentration in diet. The study indicated significant effect of Zn supplementation on ovarian follicular development with greater number of large follicles with 140 ppm Zn supplementation.

Keywords: buffalo heifers, *Bubalus bubalis*, ovarian folliculogenesis, Zn supplementation

INTRODUCTION

Zinc (Zn) influences various biological functions by being a cofactor for more than 300 metalloenzymes (Chasapis *et al*., 2012). Besides this, Zn is essential for immune responses (Gruber *et al*., 2013) and also plays an important role in antioxidant defense system (Oteiza, 2012). Similarly, Zn is required for female reproductive system and necessary for normal ovulation and fertilization (Tian and Diaz, 2011). It protects the

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female reproductive system from reactive oxygen species by lowering the oxidative stress (Agarwal *et al*., 2012). In addition to this, several researchers have noticed beneficial effect of higher levels of Zn supplementation than recommended levels on reproduction (Laar and Jongbloed, 2010; Monem and El-Shahat, 2011).

At field level, farmers shows much emphasis on balancing of protein, carbohydrates, fibre and fat fractions in diets of animals to attain higher performance but less attention is focused on the mineral specifications, especially the trace minerals that function as cofactor for metalloenzymes and influencing the metabolism of the animals at cellular level. Borderline minerals deficiencies primarily influence animal reproduction performance, mainly ovarian folliculogenesis, maturation and development of oocyte, fertility before expressing the clinical symptoms (Ashworth and Antipatis, 2001). In addition, the micronutrient requirement changes with the physiological state of the animal. Asia is having 192.23 million buffaloes contributing 97.04% of world buffalo population (FAO STAT, 2012) and accounting for about 97.07% and 88.06% of the world's buffalo milk and meat production, respectively (FAO STAT, 2012). In spite of such a huge population and big share in livestock product industry, no separate mineral requirement standards are available for this species. The concentration of Zn in livestock feeds and fodders is critically deficient in most parts of the world (Nielsen, 2012). However, there are no reports on Zn requirement standards for buffaloes and presently values recommended for cattle are being used for this species. The research on role and effect of Zn on ovarian folliculogenesis in buffaloes is negligible. Thus the present study was undertaken to investigate the effect of dietary zinc supplementation on ovarian folliculogenesis in buffalo heifers.

MATERIALS AND METHODS

Animal and feeding management

Eighteen Murrah buffalo heifers weighing 278.7±16.82 kg body weight were randomly divided in to three groups of six animals in each in completely randomized design. All the experimental animals were housed in a well-ventilated animal shed with the provision for individual feeding and watering. Strict hygienic and management practices were followed throughout the experimental period of 150 days. A basal diet (BD) was prepared using locally available feed ingredients (Table 1) to meet the nutrient requirements of dairy cattle (NRC, 2001) except Zn. The estimated Zn content in the

Table 1. Ingredient composition (%) of basal diet.

Ingredient	Composition (%)
Sorghum straw	40
Maize	40
Soya bean meal	6.5
Molasses	8.5
Red gram chunni	1.41
Urea	1.0
Limestone powder	0.8946
Mono calcium phosphate (MCP)	1.3503
Salt	0.2086
Trace mineral and vitamin premix*	0.2079

*Trace mineral premix provided (mg/kg diet): Iron, 41; manganese, 21; copper, 10; cobalt, 0.1; iodine, 0.27; selenium, 0.3. Vitamin A, D and E were provided to supply 2927 IU; 1097 IU and 39 IU per kg diet, respectively.

BD was 29.72 ppm. Feed grade $ZnSO_4.7H_2O$ (22% Zn, Venvet Chemicals Private Limited, India) was added to the BD to supply 80 and 140 ppm of Zn in experimental diets. The animals in control group were fed with BD with no Zn supplementation, whereas the Zn supplemented groups were fed with BD+80 ppm Zn and BD+140 ppm.

Ovarian folliculogenesis in heifers

After 2 months of feeding trial, ovarian folliculogenesis study was made on all animals daily with help of B-mode linear array ultrasound scanner (Model Prosound 2, Aloka Make) equipped with 7.5 MHz transducer designed for trans rectal placement. The follicle sizes in both ovaries were measured against the in-built centimeter scale displayed on the screen on side of ultrasound image. Based on the size, the follicles were grouped as large, medium and small. The follicles measuring >8 mm were large follicles, while those measuring 5 to 8 mm and 3 to 5 mm were medium and small follicles, respectively.

Statistical analysis

The data was subjected to one way analysis of variance. The differences between the means were tested by significance using Duncan's multiple range test (Duncan, 1955). All the statistical procedures were carried out as per the procedures of Snedecor and Cochran (1980) by programming and processing in computer.

RESULTS AND DISCUSSION

Ovarian folliculogenesis is a process of the follicular development starting from the smallest primordial follicles recruited into the growth pool through primary, preantral, and antral stages to the largest Graafian or preovulatory follicles that ovulate in response to the luteinizing hormone (LH) surge. Zn is intimately involved in controlling oocyte maturation and granulosa cell function before and during ovulation. Zinc deficiency blocks cumulus cell differentiation, cumulus expansion, and follicle rupture (Tian and Diaz, 2011). In addition, Kim *et al.* (2010) observed increase in Zn content in oocyte by about 30 to 50% during *in vitro* maturation. As well as Zn affects the oocyte quality by providing an appropriate preovulatory development, modifying concentration of circulatory gonadotrophins, steroids and metabolic hormones and also the environment with in the ovarian follicle (Favier, 1992). Zn being a component in antioxidant enzymes such as superoxide dismutase, it protects the rapidly growing ovarian follicles from reactive oxygen species (Agarwal *et al*., 2012).

The effect of Zn supplementation on ovarian follicular development is presented in Table 2, Figure 1 and Figure 3. In present study small and medium sized follicles were observed in right and left ovaries of control heifers (no supplemental Zn), with average diameter of 3.46 and 6.12 mm, respectively and no large sized follicles were observed as Zn deficiency might have affected the granulosa cells functions that are essential for maturation of ovary (Tian and Diaz, 2011). With 80 ppm Zn supplementation, large size follicles (average, 9.0 mm diameter) were observed and average diameter of medium and small follicles was 5.78 and 3.48 mm, respectively. In heifers on 140 ppm Zn supplementation, all large, medium and small sized follicles were observed and the average diameters were 8.84, 6.50 and 3.24 mm, respectively. The mean numbers of small and medium follicles were not affected by the concentration of dietary zinc supplementation in both the ovaries. The number of large sized follicles was higher (P<0.05) with 140 ppm Zn supplementation in both ovaries compared to those fed on 0 ppm Zn and the number of large sized follicles with 80 ppm Zn supplementation was intermediate between the control and 140 ppm Zn supplemented groups. The number of large sized follicles was 9.57 and 5.29% in right and left ovary with 140 ppm supplemental Zn and was 5.93 and 3.98% with 80 ppm supplemental Zn respectively (Table 2). This increase in number of large follicles in heifers with Zn supplementation could improve the ovulation rate and thereby the conception rate. These reproductively efficient heifers could replace the inefficient old stocks and hence improve the herd reproductive efficiency. Similarly, Monem and El-Shahar (2011) observed increase in number of days and incidence of oestrus, number of large follicles and ovulation in ewes with 100 or 150 ppm compared to 50 ppm Zn supplementation as ZnO.

CONCLUSION

Based on the results it can be concluded that dietary Zn supplementation is essential for ovarian folliculogenesis in buffalo heifers. In addition to that higher (140 ppm) levels of Zn supplementation showed more number of large follicles compared to 80 ppm of Zn supplementation.

Table 2. Number of different follicles (%) in ovaries of heifers fed Zn supplemented diets.

Follicles	Zn supplementation (ppm)			SEM	*P*-value
	0	80	140		
Right ovary					
Small	69.16	63.18	54.05	3.055	0.124
Medium	30.84	30.89	36.37	2.615	0.639
Large	0.00[b]	5.93[ab]	9.57[a]	1.535	0.025
Left ovary					
Small	88.64	90.44	86.77	1.414	0.598
Medium	11.37	9.56	13.23	1.413	0.596
Large	0.00[b]	3.38[ab]	5.29[a]	0.872	0.031

[ab] Means with different superscripts in arrow differ significantly: P<0.05.

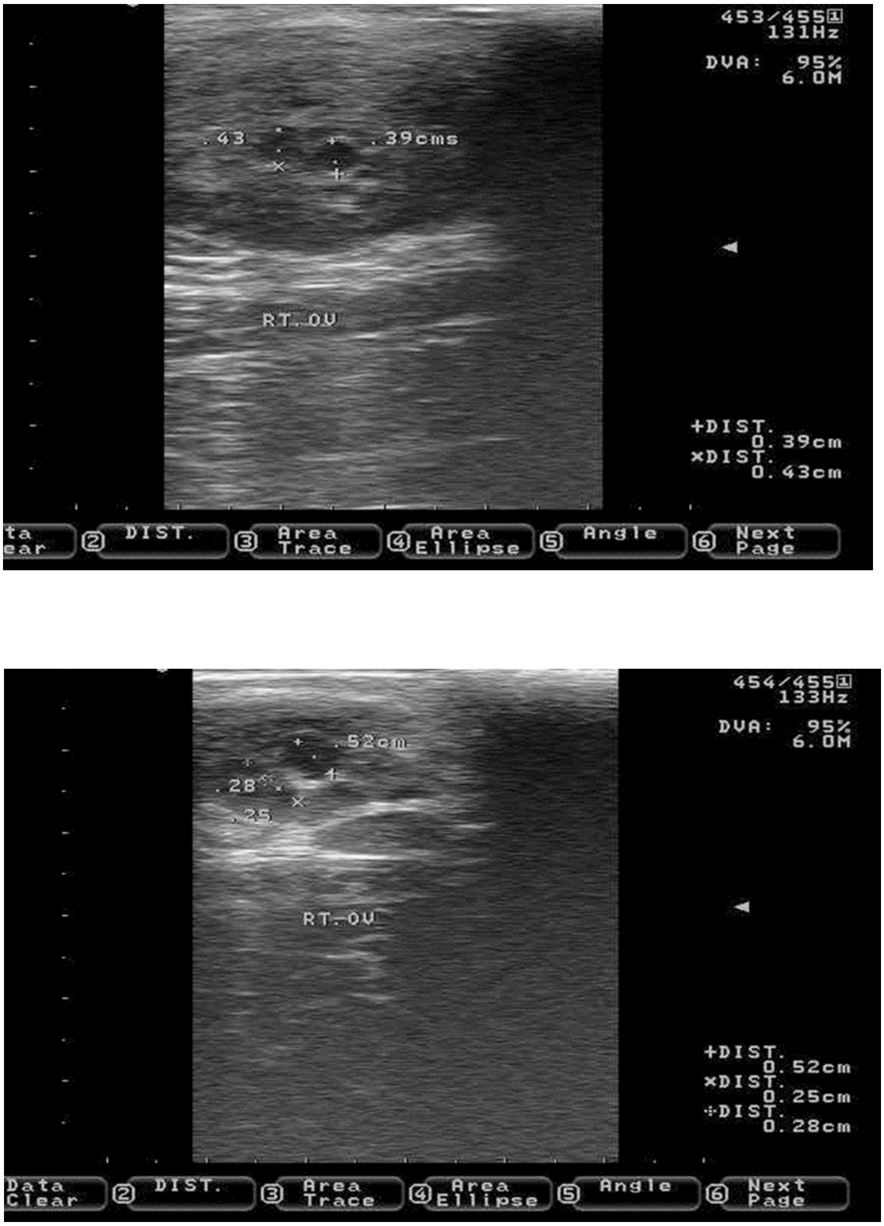


Figure 1. Ovaries of heifers fed diets without zinc supplementation (0 ppm).

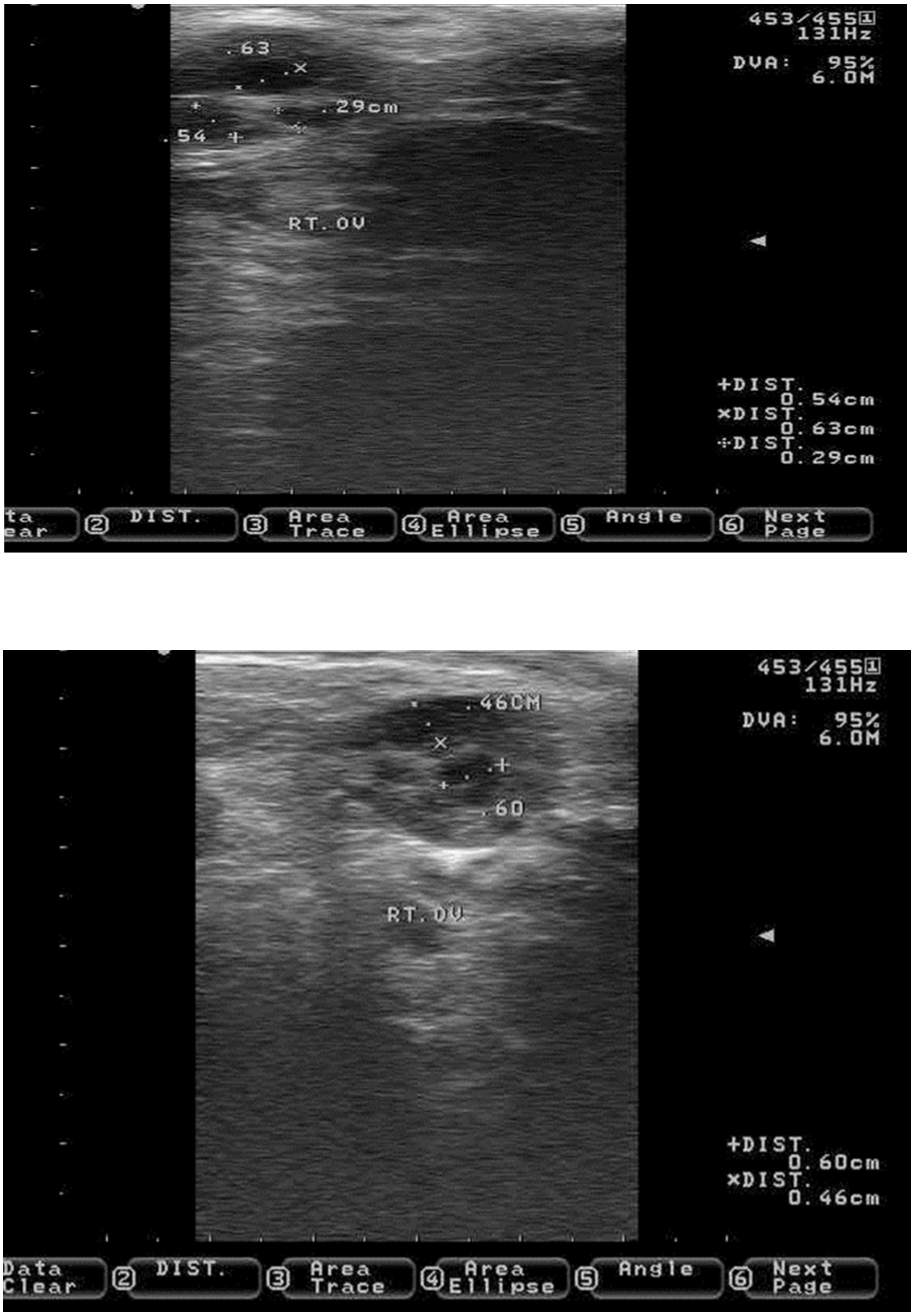
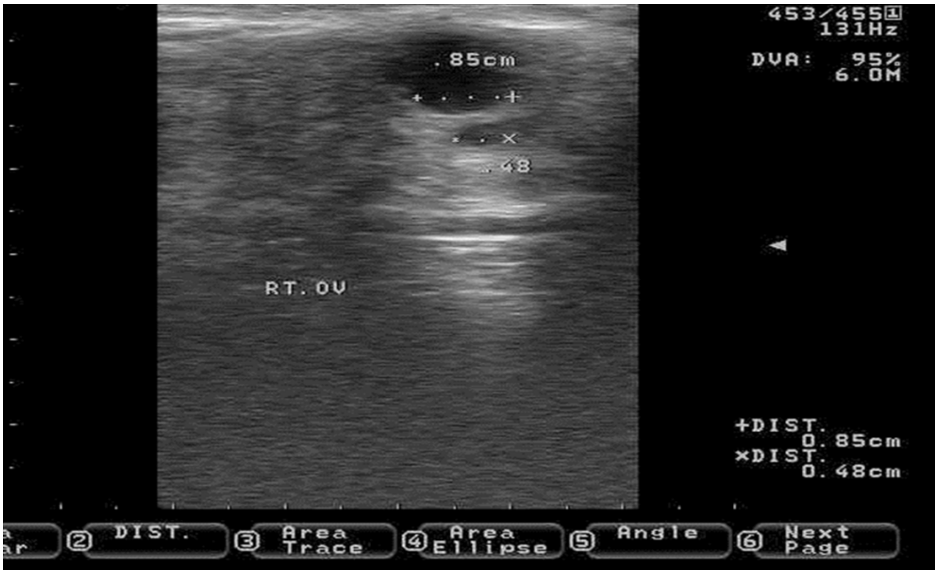


Figure 2. Ovaries of heifers fed diets supplemented with 80 ppm Zn as $ZnSO_4$.



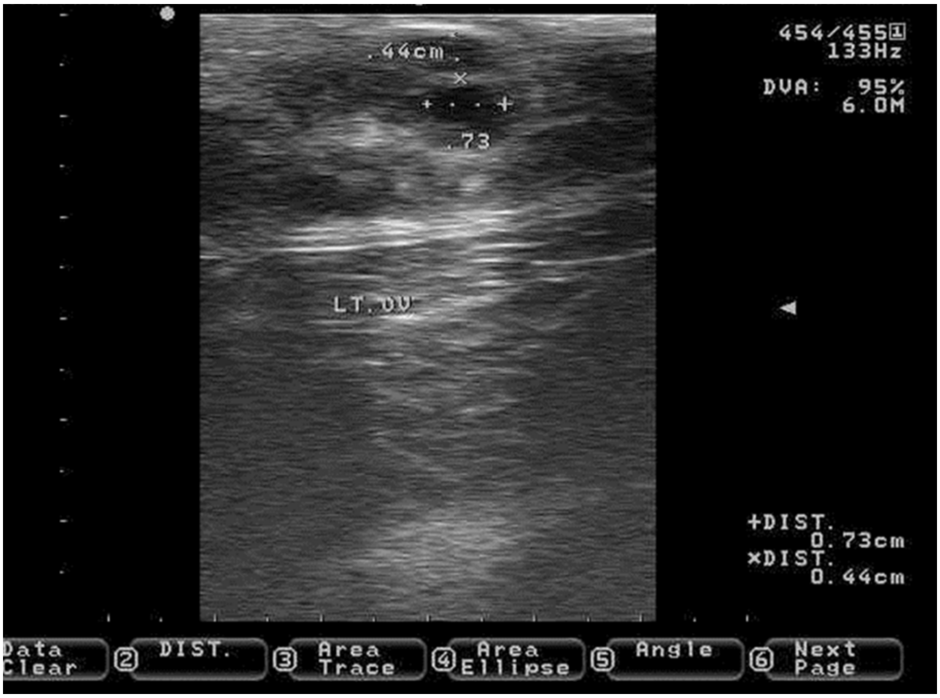


Figure 3. Ovaries of heifers fed diets supplemented with 140 ppm Zn as $ZnSO_4$.

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