



Effect of zinc supplementation on growth, reproductive performance, immune and endocrine response in grower pigs

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ABSTRACT

The effect of dietary supplementation of zinc (Zn) on growth and reproduction performances, thyroid hormones and globulin level was investigated in the present study. Gilts (24; average body weight 20.65 ± 1.72 kg) were randomly assigned into 3 groups (8 animals / group) receiving a basal diet and were supplemented for 120 days with (i) 100 mg/kg of Zn (CON); (ii) 100 mg/kg of Zn along with calcium carbonate (1.5% of dry matter of diet, to induce Zn deficiency) (DEF), or (iii) 500 mg/kg of Zn (FOR). Serum Zn level increased in FOR-fed animals, and decreased in DEF-fed compared to CON-fed. Further, DEF-fed animals developed clinical symptoms of parakeratosis. Serum Cu level was not found affected. FOR-feeding promoted tri-iodothyronine (T_3) and thyroxine (T_4) levels compared to CON-feeding. It also led to higher serum gamma globulin level. A reverse trend to FOR-feeding was observed in DEF-fed animals with respect to T_3 , T_4 and γ globulin level. Better feed conversion efficiency (FCE) and higher average daily gain (ADG) was also observed with FOR-feeding. Early puberty was attained in FOR-fed animals as compared to DEF- and CON-fed. The present study suggested that dietary supplementation of Zn at 500 mg/kg level in grower pigs might help in attaining early puberty, better growth performances, improved thyroid functions and better general health.

Key words: Estradiol, Feed conversion efficiency, Globulin, Progesterone, Thyroid hormones, Zinc

Genetics, nutrition, environment and management influence livestock production. Nutritional factors are the most crucial as directly affect the reproductive phenomenon, and have the potential to moderate the effects of other factors (Smith *et al.* 2000). Hence, there is a need to pay particular attention to the interactions between nutrition and reproduction.

One of the major factors affecting mineral nutrition in pig is the use of cereal products as the main source of diet that is lacking in many of the essential inorganic elements, as well as present in an un-utilizable form in the biological system. These have to be supplemented in biologically available forms to potentiate the desired level of bodily functions to achieve optimum growth and other productive performances. The trace minerals are powerful modulators of several physiological functions that can be considerably perturbed in deficient states (Pathak *et al.* 2011). However,

much needs to be seen to evaluate the precise role of these trace minerals on growth, reproduction and thyroid function and its interaction. Zinc (Zn) is an essential component of around 300 enzymes that are involved in a wide range of biochemical functions within the body (McCall *et al.* 2000). Zinc has a close relationship with the endocrine system; it sustains normal growth, secondary sex characteristics, reproductive function and thyroid function (Kaji and Nishi 2006). Therefore, Zn deficiency causes not only growth retardation, but also delayed sexual maturation, hypogonadism, and thyroid dysfunction. There are indications that Zn is also important for normal thyroid homeostasis and its roles are complex and may include effects on both the synthesis and mode of action of the thyroid hormones. However, it is not known whether dietary Zn deficiency has a direct effect on this aspect of thyroid hormone metabolism. In addition to its role in protein synthesis, Zn is also involved in T_3 binding to nuclear receptor (Baltaci *et al.* 2004). The genesis of the concept of Zn supplementation had come from the fact that Zn is not sufficiently stored in the body, requiring a continuous supply (Rink and Gabriel. 2001). Therefore, the experiment was designed to compare the effect of induced Zn deficiency and supplementation of Zn on certain blood biochemical

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parameters related to growth, immune and reproductive performances in grower pigs.

MATERIALS AND METHODS

Apparently healthy, crossbred (Hampshire × Assam local) gilts (24), 4-months-old (initial body weight 20.65 ± 1.72 kg), of same genetic group (75% Hampshire) were randomly selected for the experiment. Animals were maintained under uniform feeding, management and housing conditions. The animals were randomly allocated into 3 groups (CON-fed, DEF-fed and FOR-fed), each containing 8 numbers. The basal diet fed to the animals composed of maize (45%), wheat bran (21%), rice polish (10%), de-oiled groundnut cake (10%), soybean meal (7%), fish meal (5%), common salt (0.50%) and mineral mixture (1.5%) during the treatment. The animals were fed twice a day and the amount of feed offered was based upon the body weight with free access to drinking water. All the animals were humanely treated and the experiment was carried out with the due approval of Institute Animal Ethics Committee. The animals were fed the basal diet for a 7 days adaptation period, followed by experimental feeding, namely CON-fed (basal diet supplemented with 100 mg/kg of Zn, treated as control; DEF-fed (basal diet supplemented with 100 mg/kg of Zn, and calcium carbonate (CaCO_3) @ 1.5% of DM of diet to induce zinc deficiency experimentally); and FOR-fed (basal diet supplemented with 500 mg/kg of Zn). In CON- and DEF-fed groups the Zn supplementation was as per National Research Council (NRC 1998) while in FOR-fed group it is based on our hypothesis that NRC recommended level may not be sufficient for the tropical hot and humid production system. The experimental animals were fed respective diet for 120 days, and daily feed intake of each pig was recorded to calculate the feed conversion efficiency. Body weight of the experimental animals was recorded at 15-days-interval during the experimental period before feeding.

Venous blood was collected from each of the experimental animals at every 15-days-interval throughout the experimental period. Serum Zn and Cu level was estimated with the help of an atomic absorption spectrophotometer following the method of Fick *et al.* 1979. Serum concentration of tri-iodothyronine (T_3), thyroxine (T_4), estradiol 17- β and progesterone were assayed following “COAT-A-COUNT” method of RIA with commercially available kits. Serum γ globulin was estimated by the method described by Chauhan *et al.* (2006). The animals were regularly observed for visual and behavioural symptoms of estrus, and acceptance of boar for detection of age at puberty. Further, puberty was confirmed by serum estradiol 17- β and progesterone concentration.

Experimental data were analyzed using ANOVA (Snedecor and Cochran. 1994), followed by *post-hoc* comparison test employing Graph Pad Prism 4.01 software.

RESULTS AND DISCUSSION

The serum Zn concentration decreased ($P < 0.01$) in DEF-fed animals on day (d) 30 onwards of treatment as compared to CON-fed (Table 1). An opposite trend to DEF-fed was recorded in FOR-fed from d 15 till the end of study. The lowest level of serum Zn concentration observed in DEF-fed animals might be due to antagonistic effect of Ca on intestinal absorption of Zn (Lonnerdal 2000). Animals of DEF-fed group developed parakeratotic symptoms like rough hair, progressive hair loss all over the body, most prominently in the abdominal, nasal region and base of tail from d 30 onward. Animals were observed to be reluctant to eat, and the time taken to complete the feeding of the ration was more in DEF-fed compared to CON- and FOR-fed groups.

With regard to serum Cu concentration, no difference ($P < 0.01$) was observed between CON- and FOR-fed animals (Table 1) though antagonistic effect between Zn and Cu due to imbalances in their dietary level has been suggested

Table 1. Serum biochemical parameters (Mean $\pm$ SE) in different experimental groups during different periods of treatment

Parameters	Group	Period of treatment (days)									Pooled SE
		0	15	30	45	60	75	90	105	120	
Zn (ppm)	CON-fed	0.78	0.78 ^a	0.83 ^a	0.83 ^a	0.83 ^a	0.85 ^a	0.88 ^a	0.86 ^a	0.90 ^a	0.13
	DEF-fed	0.77	0.76 ^a	0.62 ^b	0.55 ^b	0.39 ^b	0.38 ^b	0.30 ^b	0.27 ^b	0.30 ^b	0.03
	FOR-fed	0.78	0.85 ^c	0.88 ^c	0.89 ^c	0.91 ^c	0.98 ^c	1.04 ^c	1.08 ^c	1.07 ^c	0.02
Cu (ppm)	CON-fed	0.83	0.83	0.85	0.85	0.86	0.86	0.87	0.87	0.87	0.01
	DEF-fed	0.83	0.83	0.87	0.85	0.86	0.87	0.87	0.87	0.87	0.02
	FOR-fed	0.82	0.83	0.84	0.84	0.85	0.87	0.87	0.87	0.87	0.03
Globulin (g/100 ml)	CON-fed	3.12	3.08	3.06 ^a	3.17 ^a	3.18 ^a	3.18 ^a	3.19 ^a	3.26 ^a	3.35 ^a	0.05
	DEF-fed	3.04	3.14	2.76 ^b	2.54 ^b	2.55 ^b	2.61 ^b	2.89 ^b	2.64 ^b	2.82 ^b	0.41
	FOR-fed	3.07	3.30	3.25 ^c	3.26 ^c	3.23 ^c	3.28 ^c	3.32 ^c	3.49 ^c	3.48 ^c	0.07
γ globulin (g/100 ml)	CON-fed	0.67	0.72 ^a	0.76 ^a	0.83 ^a	0.94 ^a	0.96 ^a	0.89 ^a	0.94 ^a	1.06 ^a	0.02
	DEF-fed	0.68	0.74 ^a	0.70 ^b	0.81 ^b	0.77 ^b	0.77 ^b	0.71 ^b	0.77 ^b	0.84 ^b	0.02
	FOR-fed	0.67 ^c	0.90 ^c	0.95 ^c	0.93 ^c	1.04 ^c	1.10 ^c	1.21 ^c	1.21 ^c	1.31 ^c	0.02

Means with different superscript within a column differ significantly ($P < 0.01$).

(Lonnerdal 2000). Neither serum Cu level was affected in DEF-fed animals, where bioavailability of Zn was made lower by adding CaCO_3 . It indicated that swine basal diet supplemented with 500 mg/kg of Zn did not impair intestinal absorption of Cu (Rincker *et al.* 2005). Serum globulin concentration decreased ($P < 0.05$) in DEF-fed animals along with the treatment periods (Table 1). A reverse trend to DEF-fed was observed in other groups during the same period which was more discernible in FOR-fed group. Serum concentration of γ globulin decreased ($P < 0.01$) in DEF-fed animals from d 30 to 120 of treatment while an increasing trend ($P < 0.01$) was recorded in CON- and FOR-fed animals from d 15 to 120 of treatment. Higher level of globulin and γ globulin recorded in CON- and FOR-fed animals might be due to Zn supplementation which has a stimulatory effect on immune system in pigs (Rupic *et al.* 1998). Further, the present study revealed that supplementation of 500 mg/kg of Zn resulted in better immunity than 100 mg/kg supplementation as reflected through serum globulin and gamma globulin concentration in FOR-fed animals. Deficiency of Zn results in lymphoid atrophy, reduced production of immunoglobulin associated with impaired T-helper cell function, reduction of thymic hormone concentration and thymus gland weight (Fernandes *et al.* 1979).

Total body weight gain at the end of experiment (animal age 8 months) was highest ($P < 0.01$) in FOR-fed animals followed by CON- and DEF-fed animals (Table 2). Besides, the lowest average daily gain (ADG) ($P < 0.05$) was recorded in DEF-fed animals. Higher body weight gain in FOR- and CON-fed groups suggests that Zn supplementation might have helped in better metabolism of feed substances. This was supported by the fact that it further resulted in higher level of metabolic hormones like T_3 and T_4 , as discussed below. Zinc is reported to be associated with the regulation of appetite by maintaining the function of taste buds (Berger 2002) and activity of carbohydrate and protein metabolizing enzymes (Jing *et al.* 2009). Additional dietary Zn in weaning diets for piglets has also been reported to result in improved growth rate and increased serum IGF-I (Carlson *et al.* 2004). The feed conversion ratio (FCR) was also better ($P < 0.05$) in FOR-fed animals as compared to CON- and DEF-fed animals (Table 2). Thus, better FCR might have supported

highest body weight gain and ADG in FOR-fed animals.

While comparing the age at puberty (Table 2), it was observed that FOR-fed gilts attained maturity at an earlier age compared to CON-fed. Further, attainment of puberty was confirmed by estimation of serum estradiol 17- β and progesterone concentration. Higher ($P < 0.01$) estradiol 17- β concentration was recorded in FOR-fed animals on d 90 of the treatment period (age 210 d) as compared to the gilts of CON- and DEF-fed groups (Table 3). This reflects the stimulatory effect of supplemented Zn on GnRH secretion to stimulate the ovarian follicular functions in FOR-fed gilts, as Zn deficiency results in lower secretion of GnRH (Underwood and Suttle 1999). The serum progesterone concentration was higher ($P < 0.01$) in FOR-fed gilts on d105 (age 225 d) of treatment indicating ovulation, which attained puberty at 218.67 ± 4.51 d. A peak progesterone level was observed in CON-fed gilts on d120 (age 240d), which attained puberty at 237.17 ± 5.27 d. Gilts of DEF-fed group attained puberty at a later age (247.00 ± 1.55 d) (Tables 2 and 3). Early age at puberty in FOR-fed gilts might be attributed to better growth rate and higher body weight gain as discussed earlier. It might have also influenced follicular development through increased synthesis and secretion of FSH and LH as Zn deficiency in female can lead to impaired secretion of both the hormones resulting ovarian dysfunction and irregular estrous cycle (Bedwal and Bahuguna 1994). No significant variation was recorded with respect to duration of estrus and length of estrous cycle among the groups.

Normal thyroid status is dependent on the presence of many trace elements for both the synthesis and metabolism of thyroid hormones. The role of zinc in thyroid metabolism has been investigated in animals but with conflicting results (Arthur and Beckett 1999; Baltaci *et al.* 2004). The serum T_3 concentration in this study revealed a decreasing trend ($P < 0.01$) from d 30 onwards till d120 in DEF-fed animals, while the FOR-fed animals registered an increasing concentration ($P < 0.01$) for the corresponding period as compared to CON-fed (Table 3). A decreasing trend ($P < 0.01$) of T_4 concentration was recorded in DEF-fed animals from d30 onward till d120 of experiment, while an increasing trend ($P < 0.01$) was recorded in FOR-fed animals from d 15 onward till d 120 of the experiment as compared to CON-fed (Table 3). The higher and lower, T_3 and T_4 concentration in

Table 2. Performance of different productive and reproductive traits (Mean $\pm$ SE) in different experimental groups during different periods of treatment

Group	Total body weight gain (kg)	ADG (kg)	FCR	Age at puberty (days)	Body weight at the age of puberty (kg)	Duration of estrus (H)	Length of estrous cycle (days)
CON-fed	38.77 ^a $\pm$ 0.85	0.31 ^a $\pm$ 0.007	3.72 ^a $\pm$ 0.09	237.17 ^a $\pm$ 5.27	51.55 $\pm$ 2.47	36.63 $\pm$ 1.12	22.33 $\pm$ 1.50
DEF-fed	30.05 ^b $\pm$ 1.20	0.25 ^b $\pm$ 0.009	4.79 ^b $\pm$ 0.17	247.00 ^b $\pm$ 1.55	49.16 $\pm$ 4.01	36.42 $\pm$ 0.99	22.25 $\pm$ 1.03
FOR-fed	43.38 ^c $\pm$ 0.66	0.36 ^c $\pm$ 0.005	3.29 ^c $\pm$ 0.05	218.67 ^c $\pm$ 4.51	50.45 $\pm$ 2.18	37.08 $\pm$ 2.89	21.83 $\pm$ 1.47

Means with different superscript within a column differ significantly ($P < 0.01$), * ($P < 0.05$).

Table 3. Reproductive and thyroid hormone profile (mean±SE) in different experimental groups during different periods of treatment

Parameters	Groups	Period of treatment (days)									Pooled SE ±
		0	15	30	45	60	75	90	105	120	
Estradiol 17-β (pg/mL)	CON-fed	25.74	25.94	26.75	27.01	26.29	27.67	25.49 ^a	25.50 ^a	31.06 ^a	1.54
	DEF-fed	25.13	25.99	25.83	26.89	25.41	26.54	25.99 ^a	25.87 ^b	25.22 ^b	1.00
	FOR-fed	26.51	26.51	26.25	27.76	26.27	27.79	30.44 ^c	35.27 ^c	24.02 ^c	2.43
Progesterone (ng/ml)	CON-fed	0.24	0.25	0.25	0.26	0.23	0.24	0.23	0.24 ^a	3.58 ^a	0.02
	DEF-fed	0.25	0.23	0.24	0.25	0.23	0.23	0.24	0.23 ^a	0.24 ^b	0.02
	FOR-fed	0.25	0.25	0.25	0.26	0.24	0.22	0.23	6.31 ^c	4.2 ^c	0.31
T ₃ (ng/ml)	CON-fed	0.75	0.72	0.74 ^a	0.73 ^a	0.73 ^a	0.76 ^a	0.77 ^a	0.74 ^a	0.75 ^a	0.02
	DEF-fed	0.77	0.76	0.65 ^b	0.63 ^b	0.64 ^b	0.57 ^b	0.54 ^b	0.41 ^b	0.43 ^b	0.02
	FOR-fed	0.73	0.89	0.88 ^c	0.88 ^c	0.94 ^c	1.13 ^c	1.17 ^c	1.19 ^c	1.45 ^c	0.01
T ₄ (ng/ml)	CON-fed	27.08	27.96 ^a	29.61 ^a	29.46 ^a	30.21 ^a	30.12 ^a	31.44 ^a	32.60 ^a	32.63 ^a	0.74
	DEF-fed	27.76	27.88 ^a	27.48 ^b	25.41 ^b	25.97 ^b	24.48 ^b	23.21 ^b	23.49 ^b	22.67 ^b	0.60
	FOR-fed	28.61	31.92 ^c	34.58 ^c	36.42 ^c	39.53 ^c	39.67 ^c	40.18 ^c	45.37 ^c	45.31 ^c	0.68

Means with different superscript within a column differ significantly ($P < 0.01$).

FOR-and DEF-fed, respectively, might be due to the fact that Zn is associated with maintaining the normal physiological status of the thyroid gland (Hartoma *et al.* 1979) and thyroid follicles (Gupta *et al.* 1997).

Our experiment revealed that higher dose of Zn supplementation (500 mg/kg) resulted in higher bio-availability of serum Zn in grower pigs. On the other hand, supplementation of CaCO₃ resulted in lower serum Zn level because of antagonistic effect and clinical manifestation of parakeratosis. Better ADG and FCR following Zn supplementation might have been due to the influence of Zn on maintenance of function of taste buds, activities of digestive enzymes, metabolic hormones (T₃ and T₄) and a healthy haematology. It is further suggested that Zn might have stimulatory effect on synthesis and secretion of FSH and LH and development of ovarian follicles, which might help in attaining early puberty. Higher serum level of globulin and γ globulin following Zn supplementation indicated its positive influence on immunity through stimulation for production of thymic hormone as well as maintenance of activities of other lymphoid organs. Thus, it may be concluded that the supplementation of 500 mg/kg of Zn in grower pigs might help in maintaining a better physiological status for achieving improved growth and reproductive performances.

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