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Effect of Dietary Supplemental Zinc on Serum Zinc, cAMP, Testosterone Concentration and Histological Architecture of Testis in Male Weanling Pig

KEYWORDS: Zinc, cAMP, testosterone, testis, pig.

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ABSTRACT *A total of fifteen numbers of weaned piglets (10.60 ± Kg body weight; 60 days of age) were used to study the supplemental effect of dietary zinc in three levels viz:-100ppm (group A), 200ppm (group B) and 300ppm (group C) consisting 5 piglets in each groups for a period of 3 months.The experimental animals were kept under intensive system of rearing as per routine standard farm managemental practices.Blood was collected from the anterior vena-cava at monthly interval from start to end of the experiment. Testis was collected at five (5) months of age by open method of castration.At 5 months of age significantly higher (P<0.05) level of serumzinc and testosterone concentration was recorded in B and C groups. As the concentration of supplemental zincincreased (A < B < C) the serum cAMPactivitiesalso found to be increased.The histological section of testis reveals that, section of B, C had compact healthy seminiferous epithelium with wide lumen packed with sperm cells.*

INTRODUCTION:- As zinc regulates more than 1000 metaloenzymes (Pallauf, 2005) it plays an important role in overall development of the biological system and more precisely in testosterone secretion (McDowell et al.,1993) and have led to their use in the form of biomarkers (Hambidge, 2003). The deficiency of zinc decreased output of pituitary gonadotropin and androgen production (Reeves and Odeel, 1988; Martin and White, 1992). Zinc also enhanced production of cAMP activity (Nishi et al.1984). Zinc has been shown to be important for normal testicular development and maintenances of the germinal epithelium (Anderson et.al., 1993). Rapidly developing testis of the young male may be particularly sensitive to inadequate amount of zinc (Prasad et.al., 1990). Therefore, the present experiment was undertaken to establish the effect of supplemental zinc on serum zinc, cAMP activities, testosterone concentration and histological architecture of testis in male weanling pig.

MATERIALS AND METHODS:- The animal experimentation was conducted at the pig farm of ICAR Research Complex for North Eastern Hill Region, Umiam, Barapani, Meghalaya and the laboratory work was done in the Department of Veterinary Physiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam. The pig farm, where the animals were maintain is located at 25° 41'21"N latitude and 91° 55'25"E longitude at an altitude of 1010msl. The agro climatic zone classified for the place is within the subtropical hill agro ecological zone. The maximum and minimum temperature normally ranges from 20.9 to 27.4° C and from 6.7 to 18.1°C, respectively. The mean annual rainfall is 2399.8 mm with relative humidity between 85 percent and 59 percent. A total of fifteen numbers of weaned piglets (10.60 ± Kg body weight; 60 days of age) were used to study the supplemental effect of zinc in three dietary levels viz:-100ppm (group A), 200ppm (group B) and 300ppm (group C) consisting 5 piglets in each groups for a period of 3 months. The experimental animals were kept under intensive system of rearing as per routine standard farm managemental practices. The experiment were designed as per the guideline of Committee for the purpose of Control and Supervision of Experiment of Animal (CPCSEA), Govt. of India and was approved by Institutional Animal Ethics Committee. Blood was collected from the anterior vena-cava at monthly interval from start to end of the experiment; serum was separated and preserved at -20 C. For estimation of serum zinc the samples were processed as per the method of Fick, et al. (1979) and were estimated using Atomic Absorption Spectrophotometer, GBC 932AA. The serum cAMP activities and testosterone concentration were estimated by using Radio Immuno Assay kits supplied by IMMUNOTECH, Czech Republic. Testis was collected at five (5) months of age by open method of castration. The representative testicular tissue samples were collected and then fixed in adequate amount of 10% formalin solution. The tissue sections of testis were processed by the method described by Dellfield as stated by Luna (1968) and stained by Hematoxylin & Eosin (Luna, 1968) for histological studies. The estimated data in the present experiment were statistically analyzed using SPSS software version 11.5.

RESULT AND DISCUSSION:- As supplemental zinc increases the serum zinc concentration also recorded significantly (P<0.05) higher in group B and C (Table 1.).

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Supplementation of zinc in the diet during the growing period results steady rise in serum zinc concentration with advancement of age (Harlikar et.al., 2000; Saikia, 2010). The rise in serum zinc concentration with advancement in age was due to reduction in growth and consequently a lower zinc requirement in the body systems (Hoekstra et.al. 1967). Many workers has reported higher serum Zinc concentration when supplemented with higher dose level of Zinc in the diet (Hill et.al.,2001; Case and Carlson, 2002 ; Buff et.al.,2005) and lower serum concentration in zinc deficient pigs (Burch et.al.,1975; Petkevicius et.al.,2003).

As presented in Table 1. the serum cAMP activities (Mean ± SE) at 2 months of age in A, B and C group were recorded as 1.83 ± 0.03, 1.21 ± 0.02 and 1.87 ± 0.04 nMol/L respectively. At 5 months of age in A, B and C group were recorded as , 18.18 ± 1.24 32.79 ± 1.11and 37.63 ± 2.74 nMol/L respectively. The Significantly (P<0.01) highest serum cAMP activities at 5 months of age was recorded in C and B group than that of the A group. An increasing trend of serum cAMP activities was observed with advancement of age this may be attributed to cAMP, which involved in signal transduction such as transferring the effects of hormones which cannot pass through the cell membrane by involving in the activation of protein kinases so, it is also known as the major communicator molecules in the living cells. Cyclic adenosine monophosphate regulates the glycogen, glucose, lipid metabolism as well as hormonal action. In the growing age the metabolic activities increases and while nearing puberty the different hormonal concentration are also found in elevated levels which might have trigger more cAMP concentration in the blood so that, cAMP will help to transfer the biomolecules inside for its specific function. As the concentration of supplemental Zn increased (A < B < C) the serum cAMP activities also found to be increased which was in agreement with the report of Nishi et al.(1984) they found that, zinc enhanced production of cAMP in rat.

The serum testosterone concentration (Mean ± SE) were 0.726 ± 0.104 ng/ml (A group), 0.654 ± 0.125 ng/ml (B group) and 0.622 ± 0.121 ng/ml (C group) at 2 months of age and at 5 months of age the values recorded was 4.77 ± 0.284, 6.54 ± 0.461 and 9.753 ± 0.730 ng/ml in A, B and C groups respectively . The serum testosterone concentration increased steadily from 2 to 5 months of age in all the groups. At 5 months of age significantly higher (P<0.05) level of serum testosterone concentration was recorded in B and C groups. Zinc appeared to have a localized affect in the testis where, the development of its capacity to produce testosterone is reduced leading to lowering in intra testicular concentration of testosterone, a critical factor for growth, development and function of the seminiferous tubules. The overall function of the testis was controlled by the gonadotropins, LH and FSH. Decreased secretion of gonadotropins was reported in Zn deficient animal (Martin and White, 1994). Zinc deficiency resulted in a lowered capacity to produced testosterone resulting in impaired testicular development (Hidiroglou and Knipfel, 1984). Zinc provides an antioxidative function and it had a positive effect on testosterone metabolism. In leydig cells of the testis, Zn ion modulates the secretion of testosterone (Mehta et.al., 1989). There are reports on positive correlation between Zn concentration and plasma testosterone concentration was observation (Kumar et. al., 2006; Devi, 2009). Zinc might have a role in functional maturation of hypothalamo – pituitary - gonadal axis mediated through different metabolic processes in the body involving various enzyme systems, more particularly, associated with carbohydrate, lipid and protein metabolism. Not only adequate supply of nutrient both macro and micro is essential for maturation of hypothalamo- pituitary- gonadal axis but proper assimilation in the system which in more important where zinc play a significant role. Present study also indicate that there was a positive correlation between serum Zn concentration and serum testosterone level indicating a major role of Zn in regulation of male reproduction.

In respect to the histological architecture of the testis a healthy seminiferous epithelium along with the seminiferous epithelial cycle was observed in group B and C (Fig. 1b,c) however the semineferous tubular section of the group A (Fig.1a) showed no such incident. The histological section of testis reveals that, section of B, C group had compact healthy seminiferous epithelium with wide lumen packed with sperm cells which was absent in group A. The present findings directly or indirectly support the earlier finding of Prasad et.al., (1990); Anderson et.al., (1993).

Conclusion:- The present experiment has established that zinc plays an important role in the comprehensive testicular development by modulating hypothalamo- pituitary - gonadal axis mediated through different metabolic processes.

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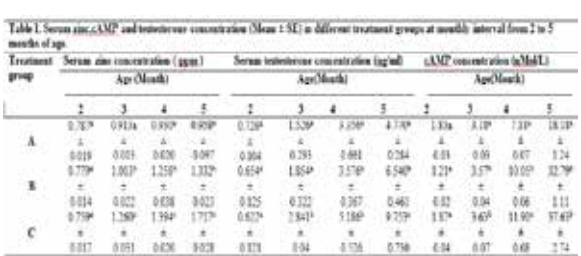

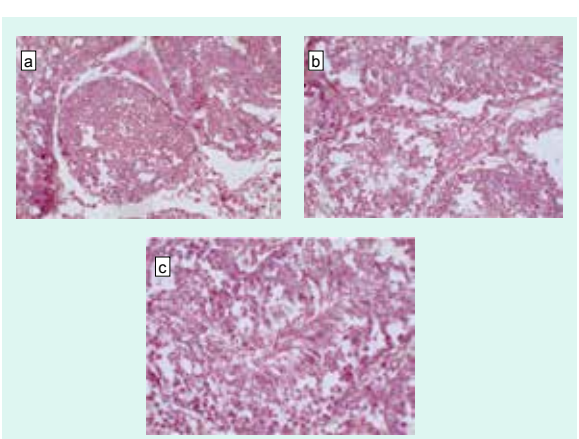

Fig, 1a,b,c : Testicular histological section of group A,B and C in pigs at 5 months of age.

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REFERENCE

Anderson, A.A., Gershwin, M.B. and Hurley, L.S.(1993). Physiology of reproduction. In : Reproduction in Farm Animals. Hafez, E.S.E., 6th Edn., W.E. Saunders, Philadelphia. | Buff, C.E., Bollinger, D.W., Ellersieck, M.R., Brommelsiek, W.A. and Veum, T.L. (2005). Comparison of growth performance and zinc absorption, retention, and excretion in weanling pigs fed diets supplemented with zinc-polysaccharide or zinc oxide. J.Anim. Sci., 83:2380-2386. | Burch, R.E., Wiliums, R.Y., Hahn, I.K., Jetton, M.M. and Sullivan, I.F. (1975). Serum and tissue enzyme activity and trace element content in response to zinc deficiency in the pig. Clin. Chem., 21 (4) : 568-577. | Case,C.L.,and Carlson,M.S. (2002).Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. Journal of .Anim.sci.80:1917-1924. | Devi, J.(2009)PhD Theses.Assam Agricultural University ,Khanapara, Guwahati,Assam. | Fick, K. R., Mc Dowell, L. R. Miles. P. H., Wilkinson, N. S, Funk, J. D. and Conrad, J. H.(1979). Methods of mineral analyses for plant and animal tissues 2nd ed. Univ. Florida, Gainesville. | Hambidge, M. (2003). Biomarkers of trace mineral intake and status. The J. Natr. 948S – 955S. (downloaded from jn nutrition.org) | Harlikar, M.N.; Tavelkar, B.A.; Deshmukh, B.T.; Nagvekar, A.S. and Ingole, S.D.(2000). Trace elements profile during growth in crossbred calves and heifers. Indian J. Anim. Sci., 70 (11) : 1147-1149. | Hidiroglou, M. and Knipfel, J.E. (1984). Zinc in mammalian sperm : A review. J. Dairy Sci., 35 (1) : 1175-1185. | Hill, G.M., Mahan, D.C., Carter, S.D., Cromwell, G.L.,Ewan, R.C., Harrold, R.L. Lewis, A.J. Miller, P.S. Shurson, G.C. and Veum, T.L. (2001). Effect of pharmacological concentration of zinc oxide with or without the inclusion of an antibacterial agent in nursery pig performance. J. Anim. Sci., 79: 934-941. | Hoekstra, W.G. Lewis, P.K. (Jr); Phillips, P.H. and Grummer, R.H. (1956). The relationship of parakeratosis, supplemented calcium and zinc content of certain body component of swine.J.Anim. Sci., 15:752-762. | Kumar, N., Verma, R.P., Singh, L.P., Varshney, V.P. and Dass, R.S. (2006). Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (Bos indicus x Bos Taurus) bulls. Reprod. Nutr. Dev., 46 (6) : 663-675. | Luna, L.G. (1968). Manual of Histological Staining Methods of Armed Forces Institute of Pathology. 3rd Edn., McGraw Hill Book Co., New York. Pp. 153-173. | Martin, G.B. and White, C.L. (1992). Effect of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. J. Reprod. Fert.,96 : 497-507. | McDowell, L.R.; Conrad, J.H. and Hembry. (1993) Minerals for grazing ruminants in tropical regions. 2nd Edn., Animal Science Department, University of Florida,. | Mehta, U., Mehta, S.N.,Georgie, G.C., Mehta, S., Dixit, V.P., Verma, P.C. (1989) Effect of dietary zinc and copper on peripheral blood plasma cholesterol, testosterone and histomorphology of testes in rats. Indian J. Exp. Biol., 27 : 469-471 | Nishi Y., Hatano S., Aihara K (1984) Effect of zinc ion on human chorionic gonadotropin stimulated in vitro production of cAMP and testosterone by rat testis. Pediatr. Res. 18: 232. | Petkevicius, S.,Darwin, K.M. and Larsen, T. (2003). Development of a low-zinc pig model for immunological investigations. Veterinarija Ir Zootechnika, 23: 45. | Prasad, A.S., Miale, A.Jr., Farid, Z., Sandstead, H.H. and Schulert, A.R. (1990). Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism and hypogonadism. J. Lab. Clin. Med., 116 : 737-749. | Pallauf, J., (2005) The importance of trace elements in animal nutrition, EMFEMA Symposium, Brussels,: inst. of animal nutrition and nutrition physiology, Justus Liebig Univ Giessen, Germany. | Reeves, P. G. and Odeel, B. L.(1988) Zinc deficiency in rats and angiotensin converting enzyme activity comparative effects on lung and testes. J. Nutr. 118 (1):622-626 | Saikia, A.(2010) M.V.Sc. Thesis, Assam Agricultural University, (Khanapara, Guwahati, Assam, |