

INFLUENCE OF Zn SUPPLEMENTATION (ORGANIC AND INORGANIC) ON BLOOD TESTOSTERONE PROFILE OF MURRAH BUFFALO BULLS

Biswajit Roy^{1,*} and P.K. Nagpaul²

ABSTRACT

The study was designed to find out the effect of zinc (Zn) supplementation (organic and inorganic) on blood testosterone profile of the Murrah buffalo bulls. The experiment was conducted over five month's periods (July to November). Twelve Murrah bulls (4 in each group) were randomly allotted to three groups with four in each group. Control group (CONT) did not receive any supplementation, whereas, ING group receive 40 ppm Zn as ZnSO₄ (inorganic Zn) and ORG group receive 40 ppm Zn as Zn-propionate (Organic Zn). Other than Zn supplementation, the diets were same for all the bulls. Blood testosterone level was not significantly ($P>0.05$) influenced by Zn supplementation. Although, higher blood plasma testosterone level (ng/mL) were observed in the Zn supplemented groups (1.08 ± 0.10 in ING and 1.02 ± 0.08 in ORG) over control group (0.93 ± 0.09). No clear pattern of monthly hormonal variation was observed. The overall results suggest that 40 ppm dietary (organic and inorganic) Zn supplementation resulted in non-significant change in blood testosterone profile.

Keywords: buffaloes, *Bubalus bubalis*,

supplementation, Murrah buffalo, blood, testosterone

INTRODUCTION

Tropical countries have reported definite or probable Zn deficiencies on the basis of clinical signs and/or low serum and forage concentrations of this element (McDowell, 1985). Studies indicated that 48.6% of the Indian soil is deficient in Zn (Gupta, 2005). Incorporation of high yielding varieties of cereals/fodders in the intensive cropping system and use of more chemical fertilizers has resulted in more areas showing deficiency of microelements status, of which Zn is important one and thus need to be supplemented. As feeding standards are generally based on the female performance, it is possible that the requirements of male animals are underestimated. In mature and older bulls, reproductive function has a higher priority than body maintenance. Although, it is difficult to define the nutrient essential to normal spermatogenic function.

The use of organic minerals in livestock nutrition has gained considerable interest over the past decade. Some studies have suggested that

¹College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh, India,
*E-mail: drbiswajitroy@gmail.com

²Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal, Haryana, India

organic Zn in ruminant diets may be metabolized differently upon absorption compared with inorganic Zn. Others obtained contradictory results. In contrast, other studies reported that there was no difference in bioavailability (Spears, 1996). However, limited research suggests that post-absorptive metabolism of organic trace minerals may differ from inorganic forms (Eckert *et al.*, 1999).

The important role of Zn in male fertility has been recognized for many years. Zn is essential trace element for the action of over 200 metalloenzymes. Zn seems to play important role in physiology of spermatozoa; it has been reported to influence the process of spermatogenesis. Deficiency of Zn in the diet delays testicular development, reduce testosterone production and stops spermatogenesis (Underwood and Somers, 1969).

Considering the potential link between improved bull reproductive development and Zn, the study was designed to investigate the effect of dietary organic and inorganic Zn supplementation on blood testosterone profile of Murrah buffalo bulls.

MATERIALS AND METHODS

Twelve healthy, sexually mature and clinically normal Murrah buffalo (MU) bulls of almost similar body weight age group were selected. The duration of the experiment was from July to November, (five months). The bulls were divided into three groups on the basis of their age and body weight following completely randomized design (CRD). CONT group did not receive any supplementation. Whereas, ING and ORG groups

received 40 ppm Zn as ZnSO₄ and Zn propionate, respectively. Zinc sulfate (analytical grade) and zinc propionate (27% available Zn) were weighted as per the requirement of individual bulls and mixed with weighed amount of concentrated mixture for feeding. The amount of Zn supplementation was adjusted at fortnight interval depending upon the total dry matter intake of individual bulls.

The buffalo bulls were reared at Artificial Breeding Complex, NDRI, Karnal. The concentrate mixture (offered to the animals) contained 33% of maize, 21% groundnut cake, 20% wheat bran, 11% rice bran, 12% de-oiled mustard cake, 1% common salt and 2% mineral mixture. Institute grown green fodder was supplied throughout the experimental period along with mixture of silage during lean season. Water was available *ad lib* throughout the day. Vaccination, deworming and other herd-health programme was followed as per the farm schedule, to ensure good health.

Collection of blood

Just after second semen collection animals were taken for blood collection. Blood samples of each animal were drawn by puncture of the vena jugularis by means of a 15 ml plastic graduated metal free tube containing 0.5 M EDTA. Plasma was obtained by centrifugation (1200 g, 30 minutes) at 4°C and plasma was aspirated in the 1.5 ml micro-centrifuge tube and frozen at -20°C until analysis.

Analysis of blood sample for testosterone

The plasma samples were subjected to the estimation of testosterone using radioimmunoassay techniques.

Data were analyzed by least square analysis of variance (Snedecor and Cochran, 1989).

Table 1. Influence of inorganic (ING) or organic (ORG) Zn supplementation on monthly blood plasma testosterone (ng/mL) profile of Murrah buffalo bulls.

Month	Control	ING	ORG	Average
July	0.92±0.14	0.96±0.16	0.85±0.07	0.91±0.07
August	1.17±0.20	1.38±0.27	0.94±0.16	1.17±0.12
September	1.21±0.34	1.20±0.34	1.34±0.36	1.25±0.18
October	0.68±0.12	0.86±0.14	1.16±0.20	0.90±0.10
November	0.65±0.21	1.01±0.23	0.89±0.06	0.85±0.11
Average	0.93±0.09	1.08±0.10	1.02±0.08	1.01±0.05

RESULTS AND DISCUSSION

The mean blood testosterone levels are depicted in the Table 1. Blood testosterone level was not significantly ($P>0.05$) influenced by Zn supplementation. Nevertheless, higher blood plasma testosterone levels were observed in the Zn supplemented groups (ING and ORG) over control group. No clear pattern of monthly hormonal variation was observed.

The testis of the adult bull secretes testosterone, which is produced by the Leydig interstitial cells under the influence of LH from the anterior pituitary. The indirect determination of bull's libido, e.g., via blood hormone levels, is an attractive proposition as it has the potential to reduce or eliminate the time, labour, aesthetic or welfare concerns, which might occur with current methods of assessing sex-drive. It would also allow the assessment of bulls, which do not respond well to such testing procedures. However, attempt to link either sporadic or sequential testosterone levels with the bull's sex-drive have generally been disappointing (Price *et al.*, 1986). This may be because of the episodic nature of hormone release and the inhibiting effects of handling and restraint of the animal or may be inaccurate assessment either of hormonal status or of libido (Post *et al.*,

1987).

Spermatogenesis is a testosterone dependent process (Sharpe *et al.*, 1988). This hormone can either diffuse to the seminiferous tubule or bind to a carrier such as albumin; which takes testosterone through the lymph spaces into the seminiferous tubule. High local levels of testosterone are found surrounding the seminiferous tubules due to the close proximity of the latter to the Leydig cells. In rats, the intra-testicular levels of testosterone, necessary to maintain spermatogenesis, are estimated to be about 25 to 45% of the normal levels (Sharpe *et al.*, 1988). Testosterone is needed to initiate spermatogenesis at puberty and for the maintenance of this completion of meiosis and for the differentiation of the spermatids (Poccaia, 1994). Although several other androgens (such as dihydrotestosterone, dehydroepiandrosterone and androstenedione) are produced in small amount by the testis, testosterone seems to be the major androgen present in the blood of several species (Gustafson and Shemesh, 1976).

Zn deficiency first impairs angiotensin converting enzyme (ACE) activity, and this in turn leads to depletion of testosterone and inhibition of spermatogenesis. Defects in spermatozoa are frequently observed in the zinc-deficient rat. Zn is thought to extend the functional life span of the

ejaculated spermatozoa. Male sexual behaviour in mammalian species is associated with adequate amounts of circulating testicular androgen (Davidson, 1977). Testosterone reverses the effects of castration on sexual behaviour (Sachs and Meisel, 1988); increasing doses of testosterone produce a greater level of copulatory behaviour.

Testosterone binds to the hypothalamic preoptic area (HPOA), where testosterone is converted to estradiol by aromatase to affect masculine sexual behaviour. Androgens exhibit a distinctive pattern of uptake and binding within HPOA (Roselli *et al.*, 1985) and stimulate aromatase activity through a receptor mechanism. Castration decreases aromatase activity in the HPOA. Therefore, high libido bulls probably have relatively low aromatization to estradiol in the brain and thus, positively affect libido (Henney *et al.*, 1990). The androgen-derived estrogens of the hypothalamus seem to be important in masculine sexual behaviour (Roselli *et al.*, 1985). Circulating estrogen in males is derived from conversion of testosterone to estradiol via the aromatase reaction. Only small amounts of systemic estrogen are produced directly by the testis; most of the daily production of peripheral estrogen involves adipose tissue (Coffey, 1988). Sperm obtain Zn during their development in seminiferous tubules. Hindroglou (1979) observed that Zn deficiency severely affected final stage of sperm maturation as it decreases gonadotropins and androgen output.

Previous evidence has shown that a threshold level of testosterone was required to activate male reproductive behaviours (D'Occhio and Brooks, 1982), but predicting sexual performance of the bull based on testosterone profiles has not been successful in the past (Price *et al.*, 1986). Blood testosterone levels in the present study are comparable with the available reports

(Dixit *et al.*, 1998). The relationship between testosterone blood levels and libido or semen quality in buffalo (Dixit *et al.*, 1985) bull is not obvious. However, low testosterone levels have been a suggested a cause of scarce libido in buffalo males (Bugalia *et al.*, 1998).

In accordance with the findings of the present study, the blood testosterone concentration was reported as 1.3 ± 0.9 ng/ml in Iranian buffalo bulls (Asadpour *et al.*, 2008). In contrast to the present findings, Imam *et al.* (2009) reported that supplementation of zinc in the diet improves blood testosterone level in buffalo bulls. Zinc propionate at half the dose of zinc sulfate supplementation in the diet of buffalo bulls is more effective.

CONCLUSION

The widespread use of buffalo bull semen in artificial insemination requires harvesting the maximum number of high-quality spermatozoa per collection during the shortest amount of time. In the present study, no-significant difference in testosterone profile was found following 40 ppm dietary Zn (organic and inorganic) supplementation.

REFERENCES

- Asadpour, R., F. Rezazadeh and H. Hamali. 2008. Testosterone level in Iranian buffalo bulls and its relation with semen freezability. *J. Anim. Vet. Adv.*, **7**(12): 1559-1562.
- Bugalia, N.S., B.R. Inderjeet, S.L. Gulati Garg and S.K. Verma. 1998. Thyroid functions, testosterone profiles and preputial microbes in cow and buffalo bulls. *Indian J. Anim. Sci.*, **68**: 341-343.

- Coffey, D.S. 1988. Androgen action and the sex accessory tissues, p. 1081-1120. *In* Knobil, E. and J. Neil (ed.) *The physiology of Reproduction*. Raven Press, NY, USA.
- D'Occhio, M.J. and D.E. Brooks. 1982. Threshold of plasma testosterone required for normal mating activity in male sheep. *Horm. Behav.*, **16**(4): 383-394.
- Davidson, J.M. 1977. The neurohormonal bases of male sexual behavior, p. 225-254. *In* Greep, R.O. (ed.) *Reproductive Physiology II*, University Park Press, Baltimore, MD, USA.
- Dixit, N.K., S.P. Agarwal, V.K. Agarwal and P.H. Dwarkanath. 1985. Seasonal variation in serum levels of steroid hormones and their relation with seminal quality and libido in buffalo bulls. *Theriogenology*, **24**(3): 293-303.
- Dixit, V.D., B. Singh, P. Singh, G.C. Georgie, M.M. Galhotra and V.P. Dixit. 1998. Circadian and pulsatile variations in plasma levels of inhibin, FSH, LH and testosterone in adult Murrah buffalo bulls. *Theriogenology*, **50**(2): 283-292.
- Eckert, G.E., L.W. Greene, G.E. Carstens and W.S. Ramsey. 1999. Copper status of ewes fed increasing amounts of copper from copper sulfate or copper proteinate. *J. Anim. Sci.*, **77**(1): 244-249.
- Gupta, A.P. 2005. Micronutrient status and fertilizer use scenario in India. *J. Trace Elem. Med. Bio.*, **18**: 325-331.
- Gustafson, A.W. and M. Shemesh. 1976. Changes in plasma testosterone levels during the annual reproductive cycle of the hibernating bat, *Myotis lucifugus lucifugus* with a survey of plasma testosterone levels in adult male vertebrates. *Bio. Reprod.*, **15**: 9-24.
- Henney, S.R., G.J. Killian and D.R. Deaver. 1990. Libido, hormone concentrations in blood and semen characteristics in Holstein bulls. *J. Anim. Sci.*, **68**: 2784-2792.
- Hindrogrou, M. 1979. Trace element deficiencies and fertility in ruminants: A review. *J. Dairy Sci.*, **62**: 1195-1206.
- McDowell, L.R. 1985. *Nutrition of Grazing Ruminants in Warm Climates*. Academic Press, New York, USA.
- Poccaia, D. 1994. Intercellular signaling systems. *In* Poccia, D. (ed.) *Molecular Aspects of Spermatogenesis*, R.G. Landes Company, Austin, TX, USA.
- Post, T.B., H.R. Christensin and G.W. Seifert. 1987. Reproductive performance of and productive traits of beef bulls selected for different levels of testosterone response to GnRH. *Theriogenology*, **27**: 317-328.
- Price, E.O., L.S. Kart, G.P. Moberg and S.J.R. Wallach. 1986. Inability to predict sexual behaviors by plasma concentrations of testosterone and luteinizing hormone in Hereford bulls. *J. Anim. Sci.*, **62**: 613-617.
- Roselli, C.E., L.E. Horton and J.A. Resko. 1985. Distribution and regulation of aromatase activity in the rat hypothalamus and limbic system. *Endocrinology*, **117**: 2471-2477.
- Sachs, B.D. and R.L. Meisel. 1988. The physiology of male sexual behavior, p. 1393-1486. *In* Knobil, E. and J. Neil. (eds.) *The Physiology of Reproduction*, Raven Press, New York, USA.
- Snedecor, G.W. and W.G. Cochran. 1989. *Statistical Method*, 6th ed. The Iowa State University press, Ames, Iowa, USA.
- Spears, J.W. 1996. Organic trace minerals in ruminant nutrition. *Anim. Feed Sci. Tech.*, **58**: 151-163.

Underwood, E.J. and M. Somers. 1969. Studies of Zinc nutrition in sheep. I the relation of zinc to growth, testicular development and spermatogenesis in young rams. *Aust. J. Agr. Res.*, **20**: 889-897.