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Advancement of puberty and enhancement of seminal characteristics by supplementation of trace minerals to bucks

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ABSTRACT

Attainment of puberty in animals is dependent on their age, body weight, nutritional status, genetic and environmental conditions. Nutritionally, organic minerals are suggested to improve semen production, sperm motility and male fertility. In this context, role of organic zinc (Zn) and copper (Cu) in advancing male puberty and semen characters in Osmanabadi goats were studied. Forty one (n = 41) bucks (Aged 5 months) were divided into ten groups and the dietary treatments comprised of a control group (basal diet; without additional trace mineral supplementation) and nine treatment groups that received, in addition to the basal diet, various doses of trace minerals (mg) on per kg dry matter basis, organic Zn as low Zn20, medium Zn40 and high Zn60, organic Cu as low Cu12.5, medium Cu25, high Cu37.5 and combination of organic Zn + Cu as low Zn20 + Cu12.5, medium Zn40 + Cu25, high Zn60 + Cu37.5, respectively fed for a period of 8 months. Bucks fed organic trace minerals reached puberty 28–35 days earlier than control group. In addition, improvement ($P < .01$) in testosterone hormone (ng/ml) levels (control: 1.63 ± 0.07 VS Zn60: 2.54 ± 0.02; Cu12.5: 6.17 ± 0.05; Cu25: 3.01 ± 0.04; Cu37.5: 2.39 ± 0.06; Zn20 + Cu12.5: 1.94 ± 0.02; Zn60 + Cu37.5: 2.44 ± 0.16 at 240 days), semen production capacity (sperm concentration, volume, mass motility) and semen quality (higher progressive motility, velocity, sperm membrane integrity and acrosome integrity) were observed in supplemented groups ($P < .05$) than the control bucks. The present study demonstrated that, additional feeding of organic Zn and Cu to growing male goats advanced onset of puberty and improved quantitative and qualitative semen characteristics. The results also implied that the organic Cu had a significant effect on overall performances of bucks as compared to Zn alone or Zn and Cu in combination.

1. Introduction

Puberty in male is commonly defined as the point of time when it first produces sufficient spermatozoa to fertilize a female. Attainment of puberty in small ruminants depends on their age, body weight, nutritional status, genetic and environment conditions [1]. Most bucks reach puberty when they reach 60% of mature body weight [2]. The pubertal period is associated with rapid testicular growth, changes in LH secretory pattern, a gradual increase in blood testosterone, and the initiation of spermatogenesis and onset of puberty [3].

Organic minerals are found to be more efficiently utilized in the body for optimum productive function and have been suggested to improve semen production, sperm motility and male fertility [4,5]. Among the trace minerals, copper (Cu) and zinc (Zn) elements in the diets of animals have more influence in livestock production [6,7]. Dietary deficiencies and imbalances of these minerals can result in poor growth, failure of reproduction and many other disorders [8]. Zn is essential for production of many hormones including testosterone, gonadotrophin releasing hormone, production of an antibacterial compound from the prostate gland and for attachment of the head to tail in spermatozoa [9,10]. Requirements of Zn are greater for testicular growth, development and for spermatogenesis than for body growth and appetite [11,12]. Addition of Zn to the animal diet increased daily sperm production and improved the percentage of normal and intact membrane

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spermatozoa [4,11,13]. Zn has antioxidative properties and reduces the reactive oxygen species resulting in improved fertility [14]. Cu is essential for enzyme functions, immunity, connective tissue metabolism and iron metabolism along with other functions. Reproductive efficiency is reduced due to alterations of enzyme systems caused by low Cu levels and results in reduced libido and infertility [3,15,16]. Zn along with Cu also influences reproduction indirectly through hoof and joint health [1].

Several laboratories are using latest methods like analyzing the spermatozoa transcripts, genomic markers, developmentally regulated genes and seminal plasma proteins, for predicting the sperm functional characteristics and male fertility [17–20]. These kinds of approaches may provide deep insight into the role of minerals in puberty onset, spermatogenesis and fertility. Plasma membrane integrity, acrosome intactness and viability of spermatozoa directly predict fertilizing potential and trace minerals supplementation is found to have prominent role in formation and maintenance of the structure and the function of spermatozoa [3,12]. Reports on mineral supplementation in goats are scanty, and mineral requirements for male fertility has not been studied. Hence, the present study was carried out to investigate the role of trace minerals organic Zn and Cu on advancing the puberty and to assess the changes in trace mineral level in blood and the seminal characteristics in terms of quantity and quality in male bucks.

2. Materials and methods

2.1. Feeding management of bucks

This study was conducted with the approval from institutional animal ethics committee (IAEC, ICAR-NIANP, Bengaluru). The experiment was conducted between December 2015 and August 2016. Five months old indigenous bucks (n = 41) were selected for this study and were randomly divided into ten groups (each group contained 4 bucks except the control group with 5 bucks). The feed consisted of the concentrate mixture and basal roughages at 1:1 proportion. The bucks were fed as per the Indian Standards (Nutritional requirement of livestock and poultry and Indian Council of Agricultural Research recommendation) [21]. The concentrate comprised of maize 44%, wheat bran 20%, ground nut cake (GNC) 17%, soya bean meal (SBM) 16%, mineral mixture 2% and salt 1%. The chemical composition of basal diet was: concentrate mixture/ragi straw: NDF (g/kg) 569.1/657.8, ADF (g/kg) 86.6/386.6, ADL (g/kg) 20.1/48.8, Ether extract (g/kg) 9.3/10.5, Crude protein (g/kg) 212.5/63.4, Dry matter (g/kg) 936.1/940.1, Organic matter (g/kg) 929.2/894.6, Total ash (g/kg) 70.8/105.4, Zinc (mg/kg) 21.58/10.72, Copper (mg/kg) 24.93/10.21, respectively.

For treatment animals, commercially available organic minerals (Bioplex Zn and Bioplex Cu- Alltech Pvt. Ltd. Bengaluru) were fed orally (folded in a paper) every day in the morning h until the completion of experimental period (240 days). The organic Zn treatment groups were supplemented with 3 different doses of Zn *i.e.* low Zn20, medium Zn40 and high Zn60 mg/kg DM, respectively. The organic Cu treatment groups were supplemented with 3 different doses of Cu *i.e.* low Cu12.5, medium Cu25, and high Cu37.5 mg/kg DM, respectively. The combination of organic Zn and Cu were given in 3 different doses *i.e.* low Zn20 + Cu12.5, medium Zn40 + Cu25 and high Zn60 + Cu37.5 mg/kg DM, respectively and control animals were maintained without any mineral supplementation, over the basal diet.

2.2. Body weight and puberty

Body weights (in Kgs) were recorded at fortnightly intervals using electronic weighing balance, starting on day 0 (i.e the day of starting of feeding). However, weights recorded on 0, 60, 120, 180 and 240 days of treatment were considered for analysis. Attainment of puberty was monitored by stimulating each animal with electroejaculator weekly once for semen ejaculation [3].

2.3. Blood collection and separation of plasma

Blood samples (in the morning h before feeding) were collected at fortnightly intervals starting on day 0. However, samples collected on 0, 60, 120, 180 and 240 days of treatment were considered for analysis. The blood samples were collected by jugular vein puncture in heparinized tubes and centrifuged at 4000 × g, at 4 °C for 20 min. Aliquots of plasma for trace minerals (copper, zinc) and hormones (Testosterone, T_3 and T_4) analysis were stored separately at −20 °C.

2.4. Testosterone, T_3 and T_4 estimation

T_3 and T_4 hormone levels (ng/ml) were estimated by Radioimmunoassay (RIA) using diagnostic kits (Immunotech, France) having sensitivity of 0.319 ng/ml for T3 and 12.98 ng/ml for T4. The intra and inter assay coefficients of variation were within 10%. The testosterone levels (sensitivity 0.02 ng/ml) were estimated using the Enzyme linked immuno sorbent assay (ELISA, Standardized at ICAR-NIANP Bengaluru) with an intra and inter assay co-efficients of variation maintained at 5.62% and 8.22%, respectively.

2.5. Quantification of Zn and Cu

Zn and Cu levels were estimated in blood plasma (mg/ml) by Inductively Coupled Plasma Mass spectrophotometry (ICP-OES, model: Optima 8000, Perkin Elmer, USA).

2.6. Semen collection and sperm characteristics analysis

A total of four hundred ejaculates (40 × 10 = 400) were collected from bucks by electro ejaculation method. Hemocytometer was used for determining the concentration of spermatozoa (million/ml) in the semen [22]. The semen samples were collected twice a week from each buck with three days intervals from the age of 9–13 months.

2.6.1. Volume

The volume of semen was recorded (in ml) directly from the graduated semen collection cup immediately after collection.

2.6.2. Mass motility

The mass motility or mass activity of the neat semen was assessed according to the method described by Evans and Maxwell [23]. Briefly, a drop of neat semen was placed on a clean glass slide maintained at 37 °C and edge of the drop was observed at low magnification (10 × objective) on the thermally controlled stage of phase contrast microscope. The mass motility was graded from 0 to 5 based on waves and eddies.

2.6.3. Percent live spermatozoa

Eosin-Nigrosin stain was used for determining the percentages of live and dead spermatozoa as described [24]. Minimum of 200 spermatozoa were counted to analyse live and dead spermatozoa percentage at 40 x using phase contrast microscope (Nikon Eclipse E 500, Nikon, Japan).

2.6.4. Hypoosmotic swelling and giemsa test (HOS-G test)

HOS-G test was carried out as earlier described [25]. In brief, 10 μL of neat semen was added to 200 μL of 125 mOsm (hypo-