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Effect of dietary supplementation of selenium and *Moringa oleifera* leaves on growth performance of pre-pubertal Kundhi buffalo bull calves

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Authors' Contribution | **Vighio, S.A**. has conducted the experiments, **N.A. Korejo & A. A. Memon** conducted making design, **P. Khatri** rared animals, **G. A. Mughal**, **R.A. Korejo, H. Malhi, S. Mangrio** & **L. Lakhani** collected ana analysed the data.

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ABSTRACT

The Kundhi buffalo, a unique and robust breed of water buffalo (Bubalus bubalis), is native to the Sindh province in Pakistan. This breed is well-adapted to the local environment and known for its excellent milk production, meat quality, and draught power. There is a growing need to develop strategies that focus on the sustainable conservation and genetic improvement of this breed to preserve its valuable traits for future generations. Twelve young bull calves belonging to elite Kundhi buffaloes (observed phenotypically) were obtained from progressive farmers based on their parent's performance records. The bull calves, aged approximately 10-15 months, were initially weighed (rages from 150 to 200 kg) and randomly divided into three groups, namely C, S, and M, each group were comprising 04 animals. Group C was designated as the control group and fed basal diet only, while calves belonging to Group S were given a dietary supplement of Selenium Yeast Sel-Plex®, with a dosage of 0.3mg/kg dry matter and Group M was treated with *Moringa olifera* leaves (dried) at the rate of 100g/ animal. This experiment was conducted for 12 weeks. The results of present study indicated that significantly (P<0.05) higher monthly body weight gains (14.05 kg/month) were recorded in Kundhi buffalo bull calves of S followed by the M (11.67 kg/month) in comparison to the C group (9.75kg/month) of the animals. The positive coefficient of correlation was seen between age and body weight in all tested subjects. In terms of the body conformation; the monthly developmental gains of the heart girth, body length, and height were observed significantly (P< 0.05) higher (11.2, 8.89 and 8.94 cm) in S, followed by M (8.23, 4.89 and 6.39 cm) in comparison to the control bull calves (4.74, 3.58 and 3.27 cm), respectively. High levels of the testosterone (0.481 ng/ml) were determined in the bull calves of S, followed by M (0.33 ng/ml) in comparison to C (0.208 ng/ml) group. The monthly gains of the scrotal circumference were recorded significantly (P<0.05) higher (1.65 cm) in the S, than to M (1.21 cm) and that of control (0.40 cm) group calves.

Keywords: Kundhi buffalo, selenium, *Moringa oleifera,* scrotal circumference, testosterone.

INTRODUCTION: Organic selenium is a form of the essential trace element, which is required various biological functions in humans and animals. Selenium plays a vital role in maintaining overall health, including supporting immune function, thyroid hormone metabolism, DNA synthesis, and antioxidant defense systems (Fairweather-Tait *et al.*, 2011). Organic selenium refers to selenium that is covalently bound to carbon within organic compounds, such as selenomethionine and selenocysteine. These organic forms are generally more bioavailable and less toxic than inorganic forms, such as selenite and selenite (Schrauzer, 2000). Selenomethionine, for example, is a naturally occurring amino acid that can be readily absorbed and incorporated into proteins in place of methionine, thereby enhancing the bioavailability of selenium (Rayman, 2000). Organic selenium is commonly found in food sources such as Brazil nuts, whole grains, fish, and meat. Selenium content in plant-based foods can vary widely, depending on the selenium concentration in the soil where they are grown. Selenium is a vital micronutrient that plays a crucial role in the reproductive health of cattle and buffalo. It is involved in various physiological processes, including the metabolism of thyroid hormone, immune function and antioxidant defence systems. Selenium deficiency or suboptimal levels can lead to reproductive disorders and decreased fertility in both male and female livestock (Mehdi and Dufrasne, 2016). In male cattle and buffalo, selenium is important for maintaining testicular function, sperm production, and overall fertility. It is an integral part of the glutathione peroxidase enzyme, which protects sperm from oxidative damage and ensures their viability and motility (Sordillo and Aitken, 2009). Selenium deficiency in males can lead to poor semen quality, reduced sperm motility, and increased morphological abnormalities, ultimately reducing fertility (Mehdi *et al.*, 2013). In female cattle and buffalo, adequate selenium levels are essential for maintaining normal ovarian function, successful conception, and healthy pregnancy outcomes. Selenium deficiency has been associated with different reproductive disorders, such as delayed puberty, silent heat or estrus, reduced conception rates, and increased incidences of retained placenta, metritis, and abortion (Mehdi and Dufrasne, 2016). Supplementing selenium in the diet has been shown to improve reproductive performance and reduce the risk of these issues (Juniper *et al.*, 2008). *Moringa oleifera*, commonly referred to as the drumstick tree or horseradish tree, or simply moringa, is a fast-growing, drought-tolerant tree native to the Indian subcontinent. It is a member of the family *Moringaceae* and is now widely grown in tropical and subtropical regions across the globe due to its medicinal and nutritional properties (Anwar *et al.*, 2007). It is recognized under different aliases, including moringa, drumstick tree (owing to its elongated and slender seedpods), horseradish tree (due to the horseradish-like taste of its roots), oil tree (because of its high behenic acid content), and miracle tree (due to its medicinal attributes). Studies have shown that *M. oleifera* leaves have protective effects on spermatogonia cells and can reduce cell damage in mice injected with cyclophosphamide (Nayak *et al.*, 2016). The hexanend extract of *M. oleifera* leaves has been found to enhance the functions of somniferous tubules, epididymis, testis, and seminal vesicles in male mice (Cajuday and Pocsidio, 2010). Additionally, combining *M. oleifera* with hormone supplements has been shown to improve the maturation rate of sheep oocytes and promote the expression and synthesis of essential proteins for the maturation processes. Adding Moringa leaves to the diet of bali bulls improved the overall movement and forward movement of their sperm. The levels of total and progressive motility observed in this study were like those previously reported for the same breed of bulls at the Singosari Artificial Insemination Center (Syarifuddin *et al.*, 2017). The effects of *M. oleifera* were positive on sexual behaviour in male mice. It increased the frequency of mounting, reduced the time taken to ejaculate, and increased the frequency of successful mating. It also had a significant impact on libido and sperm count (Zade *et al.*, 2013). *Moringa oleifera* leaves contained beta-carotene and other powerful antioxidant compounds such as kaempferol, quercetin, rutin, and caffeoylquinic acids. It also contained essential antioxidant micronutrients like selenium and zinc, as well as antioxidant vitamins C, E, and A. These compounds were found to have regulatory effects on fertility performance (Vongsak *et al.*, 2014). Various fertility-enhancing properties of *M. oleifera*, including aphrodisiac effects, increased fertility, improved mating behaviour, and treatment of reproductive diseases in males. Moringa leaves supplementation in Bali bulls observed increased concentrations of testosterone in their blood plasma. During the treatment period, testosterone levels were consistently higher throughout the day, the time between approaching a female and ejaculation was shorter, and both total and progressive sperm motilities were higher compared to the period without supplementation (Dafaalla *et al.*, 2016). The early production of semen of a bull calf depends largely on the growth of the animal. The

bulls therefore should be grown out early and well, avoiding, as far as possible damage to the testicular tissue from any cause. The age at which puberty begins depends on many factors, but size for the breed seems to be the main controlling factor (Korejo *et al.*, 2019).

OBJECTIVES: The objectives of this study were to investigate the growth performance and the testosterone level of pre pubertal Kundhi buffalo bull calves under the oral supplement with Selenium and *M. oleifera* leaves.

MATERIALS AND METHODS: **Body weight:** Body weight was taken early in the morning of every bull calf before feeding. According to Schaeffer's formula (Measuring Tape Method) can be measured with following equation.

$$W = \frac{L \times G2}{300}$$

W represents the body weight in pounds, L signifies the length of the animal in centimetres, The measurement was taken from the shoulder's starting point to the pin bone, and G denotes the chest girth of the animal in centimeters.

Body conformation: The body conformation of the buffalo bull calves was taken by checking the height at wither (from wither straight to ground), length (from point of shoulder to pin bone), heart girth (circumference of chest).

Scrotal circumference: The scrotal circumference of buffalo bull calves was measured. To determine the scrotal circumference, carefully pull the testis down towards the lower part of the scrotum by looping a rubber band around its neck. Then, using a measuring tape, the circumference of the scrotum can be measured in centimeters.

Testosterone level: Early in the morning using a 10-cc syringe, Blood samples were obtained from each calf by collecting them from the jugular vein. and placed into a blood-collecting tube. To continue the process, the tubes were brought to the lab. All of the Kundhi buffalo bull calves had their blood taken on day one and again at the conclusion of the experiment. The blood samples were then separated from the serum by centrifuging them at 3000 rpm for 20 mins Subsequently, the collected blood samples were stored in sterilized glass vials at a temperature of -20 °C. These samples were preserved for later use with kits designed to measure the levels of serum testosterone. Serum-labelled testosterone was added to the standard samples and the unidentified samples to be incubated in tubes covered with antibodies. The liquid inside the tubes was aspirated after incubation, and a gamma counter was used to measure the bound radioactivity. Interpolation was used for figuring out the testosterone levels in unidentified samples by utilizing an equivalence curve prepared using six standards.

Statistical analysis: The data were processed on different statistical analysis utilizing computer program GraphPad Prism v.5 to determine the correlation coefficient and ANOVA-II between the treated groups.

RESULTS: **Body weight (Kg/month):** Results on the body weight of Kundhi buffalo bull calves fed on selenium and *M. oleifera* leaves are mentioned in figure 1.

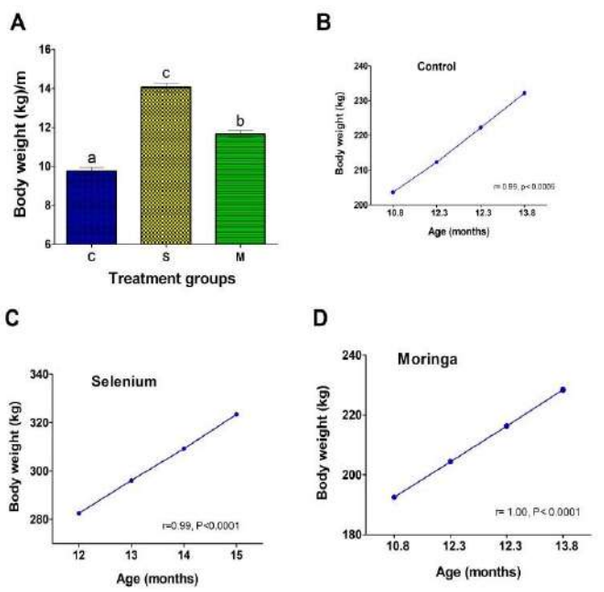

Figure 1: The average monthly gains of body weight (Kg/month) of Kundhi buffalo bull calves supplemented with selenium and *M. Oleifera* leaves in comparison to control subjects (A), Coefficient of correlation between age and body weight of a bull from control group (B), Selenium (C) and *M. Oleifera* (D).

Data indicates that significantly (P<0.05) increased body weight gains (14.05 kg/month) were recorded in Kundhi buffalo bull calves fed on selenium as compared to group M (11.67 Kg/month) fed on *Moringa oleifera* leaves. Lower body weight gains (9.75 Kg/month) werenoted in Kundhi buffalo bull calves ofthe control in comparison to the animals of S and M groups. Correlation analysis between the body weight and age was performed for all groups in individual bulls. Results showed that there was positive correlation among the body weight versus age of Kundhi buffalo bull calves. The body weight of Kundhi buffalo bull calves were increased more in selenium and *Moringa oleifera* leaves feeding group compared to that of control (figure 1).

The age, body length, heart girth, and height: The body conformation, including the heart girth, length, and height of Kundhi buffalo bull calves versus age of the animals were described in control, selenium and *Moringa oleifera* leaves feeding groups are mentioned in the table 1.

S.No:	Animals	Age (months)	Heart girth (cm)	Length (cm)	Height (cm)
1	C1	11.5 - 14.5	147.23 ± 2.72	105.32±1.95	104.65±1.86
2	C2	10.5 - 13.5	130.93 ± 3.51	108.72±2.49	112.20±1.78
3	C3	10.0 - 13.0	115.35 ± 3.39	99.65±2.45	84.32±1.90
4	C4	9.5 - 12.5	131.65 ± 2.43	111.90±2.56	109.03±2.98
5	S1	12.0 - 15.0	176.18 ± 6.66	135.00±4.84	143.75±5.47
6	S2	10.5 - 13.5	154.68 ± 7.39	123.33±5.99	121.05±5.54
7	S3	10.5 - 13.5	148.57 ± 7.07	122.33±6.55	124.52±6.30
8	S4	9.0 - 12.0	147.45 ± 7.63	116.50±5.45	118.38±5.91
9	M1	9.0 - 12.0	156.90 ± 5.02	114.45±3.41	127.07±3.82
10	M2	11.0 - 14.0	153.30 ± 5.68	112.65±3.02	130.95±3.95
11	M3	11.5 - 14.5	137.05 ± 5.21	102.67±2.93	118.75±4.19
12	M4	9.0 - 12.0	149.55 ± 5.28	115.05±3.38	107.55±4.56

Table 1: The average heart girth, body length, and height of the individual Kundhi buffalo bull calves in control, selenium and *M. oleifera* leaves within the range of different age levels (Mean ± SEM). Abbreviations C1 – C4 (control bulls), S1 – S4 (bulls fed selenium), M1 – M4 (bulls fed *M. oleifera* leaves.

The data table presents the measurements of 12 animals, categorized into three groups: C (C1-C4), S (S1-S4), and M (M1-M4). For each animal, four variables were measured: Age (in months), Heart girth (in cm), Length (in cm), and Height (in cm). Each variable is presented in the table with its corresponding mean and standard error of the mean (SEM). The animals in group C have ages ranging from 11 to 13 months and show relatively smaller heart girth, length, and height values compared to the other two groups. Animals in group S have ages between 10.5 and 13.5 months and generally exhibit larger heart girth, length, and height values. Lastly, animals in group M have ages between 10.5 and 13 months and present heart girth, length, and height measurements that fall between the values of the other two groups. The findings of this study related to the heart girth, body length, and height measurements of male buffalo calves are presented in figure 2.

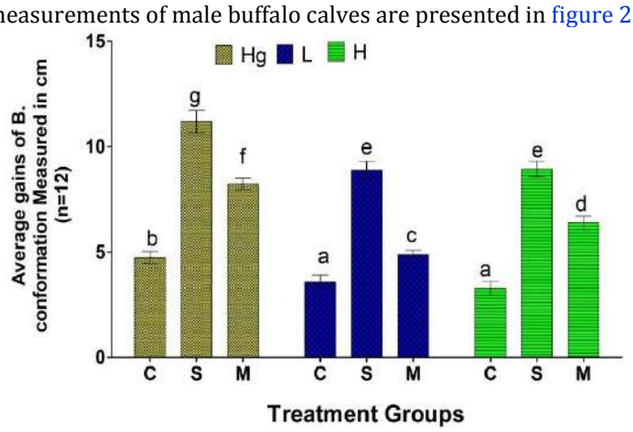

Figure 2: The average monthly gains of heart girth, body length and height of Kundhi buffalo bull calves in control animals and supplemented with Selenium and *M. olifera* leaves.

Our data indicated significantly (P<0.05) higher heart girth (11.2 cm), body length (8.89 cm) and height (8.94 cm) in Kundhi buffalo bull calves fed on selenium followed by (8.23, 4.89 and 6.39 cm) bull calves fed on *moringa oleifera* leaves compared tothe control animals (4.74, 3.58 and 3.27 cm) respectively.

Testosterone level: The testosterone level of the Kundhi buffalo bull calves fed on selenium and *Moringa oleifera* leaves compared to control subjects were recorded before and after the treatment in different group of animals and are mentioned in figure 3.

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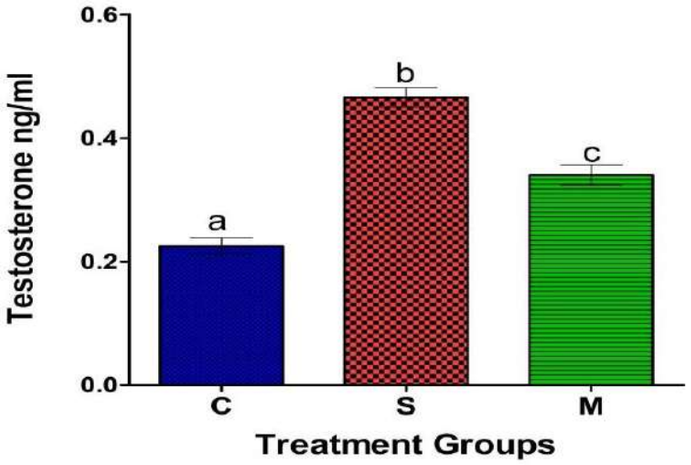

Figure 3: Average Testosterone level of Kundhi buffalo bull calves supplemented with selenium and *M. Oleifera* leaves compared with control subjects (n=04)and p value (<0.05).

The findings showed that there was no difference in the amount of testosterone that was non significant (P>0.05) i,e (0.067, 0.069 and 0.069 ng/mL) were recorded in all set groups before the start of the treatments. However, significantly (P<0.05) high level of testosterone (0.481 ng/ml) was determined in Kundhi buffalo bull calves of S group fed selenium followed by M (0.33 ng/ml) animals offered *moringa olifera* leaves compared to C (0.208 ng/mL) that of control group (figure 3).

Scrotal circumference of Kundhi buffalo bull calves: The monthly growth of the scrotal circumference of the Kundhi buffalo bull calves were observed in animals fed on selenium and *Moringa oleifera* leaves in comparison to control normal diet are mentioned in figure 4.

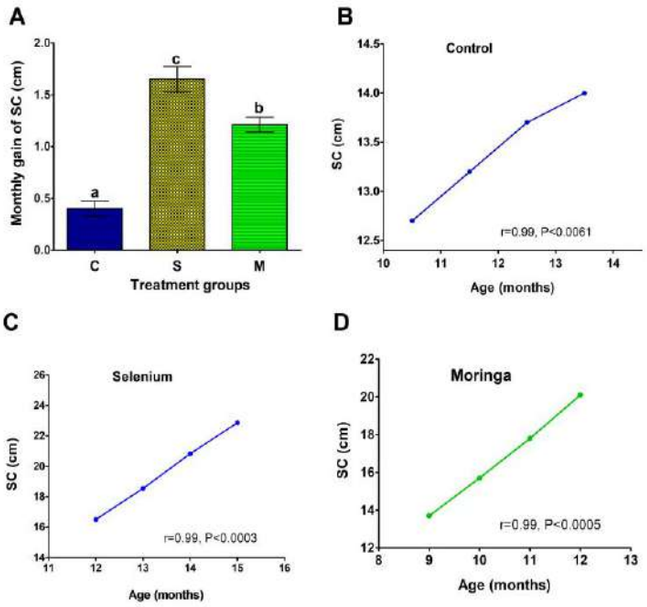

Figure 4: The average monthly gains of scrotal circumference (SC) of Kundhi buffalo bull calves supplemented with selenium and *M. oleifera* leaves compared with control aniamls (A), coefficient of correlation between age and scrotal circumference of a bull from control group (B), Selenium (C) and *M. Oleifera* (D).

The findings showed that considerably (P<0.05) higher monthly gains of the scrotal circumference (1.65 cm) were noted in group S followed by M (1.211 cm) compared to that of C (0.401 cm) group of animals. The positive correlation among the scrotal circumference vs age of Kundhi buffalo bull calves was noticed in all treated group of animals. The development of the scrotal circumference of Kundhi buffalo bull calves were found good by feeding selenium followed by *Moringa oleifera* leaves compared to control subjects.

DISCUSSIONS

The findings of the research suggested that the Kundhi buffalo bull calves who were given se and *M. oleifera* leaves as part of their diet experienced a noteworthy rise in their body weight in comparison to the group that did not receive these supplements. The results of this investigation are consistent with earlier research that highlights the positive effects of these supplements on animal growth and development. Genedy and Mohamed (2014) found that dietary se supplementation resulted in a significant increase in body weight among Egyptian buffalo calves. Similarly, the higher body weight gains were observed in lambs fed with *M. oleifera* leaves compared with the animals under normal routine diet (Moyo *et al.*, 2014). In our research, we also observed a direct correlation between the age and body weight of male buffalo calves belonging to all groups of the Kundhi breed. This result is consistent with previous research that has shown that there is a direct relationship between age and body weight in livestock animals.

Studies on goats (Mendieta-Araica *et al.*, 2011) and rabbits (Moyo *et al.*, 2011) also reported improved weight gain and health parameters with *M. oleifera* supplementation, further supporting our results. However, it's worth noting that the degree of weight gain observed in our study seems higher than in some of the previous research. This discrepancy might be due to differences in the specific nutritional needs and metabolism of buffalo bull calves compared to other species, or perhaps due to synergistic effects of selenium and *M. oleifera* when provided together. Further research would be needed to investigate these possibilities. Our study's outcomes revealed that Kundhi buffalo bull calves who were given selenium and *M. oleifera* leaves had substantially greater heart girth, length, and height as compared to the control group. The study found a significantly higher level of testosterone in the group fed with selenium compared to those fed with *M. oleifera* leaves and the control group. Interestingly,almost no research has been done to examine the impact of selenium and *M. oleifera* leaves on the testosterone levels of male buffalo calves. In that context our study has found novel outcomes of selenium and *M. oleifera* leaves on the hormonal level of testosterone. The impact of adding selenium to the diet of male pigs was explored, with a focus on how it influenced their testosterone levels. In pigs, administering selenium supplements led to a substantial elevation in the levels of serum testosterone. The influence of *M. oleifera* leaf intake on the reproductive performance of male rabbits was assessed. The study findings revealed a significant upsurge in the levels of serum testosterone due to *M. oleifera* leaf supplementation (Dafaalla *et al.*, 2016). Testosterone is a critical hormone which plays a vital role in the formation and operation of male physical traits associated with reproduction, as well as growth and muscle development. Thus, the enhanced testosterone levels in the selenium-supplemented group could contribute factors to the observed rise in body weight and physical dimensions. Scrotal circumference is often used as an indicator of reproductive potential in male livestock, as it correlates with testicular size and sperm production. Therefore, the increase in scrotal circumference suggests that selenium and *M. oleifera* supplementation could potentially enhance reproductive capacity in buffalo bull calves. Findings of current study indicated that feeding Kundhi buffalo bull calves with selenium and *M. oleifera* leaves resulted in significantly higher monthly gains of the scrotal circumference compared to the control group. Similarly, higher scrotal circumferences were reported in Nili-Ravi buffalo bulls under the supplementation of selenium in their ration. *M. oleifera* effects of intake on the development and reproductive abilities of West African Dwarf goats (Wafa *et al.*, 2017). A noteworthy rise in scrotal circumference and testicular weight in the goats that were given *M. oleifera* leaf supplements. The significance of selenium in maintaining reproductive health has been thoroughly studied in past research. Selenium supplementation in rams led to a significant increase in scrotal circumference (Ziaei, 2015), aligning with our findings. This study also suggested that the selenium-supplemented rams had a higher sperm count, indicating a potential improvement in fertility.

CONCLUSIONS: The results of present study concluded that feeding Kundhi buffalo bull calves with selenium and *M. oleifera* leaves were improved body weight, heart girth, length, height, and scrotal circumference, as well as increase their testosterone levels. The outcomes suggest that administering selenium and *M. oleifera* leaves to male Kundhi buffalo calves could have the potential to enhance their growth and reproductive abilities. Therefore, it can be inferred that these supplements may be beneficial for the management of Kundhi buffalo breeding. These findings have significant implications for livestock management practices, as improving animal growth and reproductive performance can lead to increased efficiency and productivity in animal production systems.

CONFLICT OF INTEREST: Authors have no conflict of interest

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