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SUPPLEMENTATION OF SHATAVARI *(Asparagus racemosus)* ROOT POWDER AUGMENTS GROWTH AND REDUCES THE AGE AT PUBERTY IN MURRAH BUFFALO HEIFERS

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ABSTRACT

The effects of shatavari root powder supplementation during prepubertal period on growth and reproductive performance was studied in Murrah buffaloe heifers. Twelve Murrah heifers with an average age of 18.5±0.77 months and with an average body weight of (220.05±7.11 kg) were randomly divided in two groups. Six Murrah heifers as Control group, while another six Murrah heifers served as a Treatment group which is supplemented with shatavari root powder 100 mg/kg live body weight once in the morning during experimental period. The body weight gain Treatment group in the present investigation was significantly (P<0.01) higher than the heifers of Control groups. The average daily weight gain of heifers in Treatment group was statistically (P<0.05) higher than untreated Control group. However, DMI as a percent of live body weight showed statistically significant (P>0.05) in Treatment than Control group. The difference in age at puberty between Control and Treatment groups was found to be significant (P<0.05). Whereas the difference in age at sexual maturity among between Control and Treatment group was found be significant (P<0.05). The difference in age at age at first conception among the group was found be significant (P<0.05). It was concluded that prepubertal period supplementation of shatavari significantly improves the body weight and reducing the age at puberty, in Murrah buffalo heifers.

Keywords: *Bubalus bubalis*, buffalo Heifers, Shatavari root powder, body weight, puberty

INTRODUCTION

Buffalo is known as the world second most important milch animal because its shares more than 95% of the milk produced in South Asia (Javaid *et al*., 2009) The world buffalo population is continuously increasing and was estimated more than 188.33 million out of these more than 95% (178.91 millions) of the world population is found in Asia (FAO STAT 2017). The present buffalo's population in India is 105.34 million, which is first position in the world. Buffalo, the premier dairy animal in India contributing approximately 56% of total milk produced in the country (Bhas,

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2017). However, low reproductive efficiency in general and in the buffalo in particular remains a major economic problem globally and its incidence is higher in India (Ingawale and Dhoble, 2004). Reproductive efficiency is the primary factor affecting productivity and is hampered, in female buffalo, by late attainment of puberty, seasonality of calving, long postpartum anoestrus and subsequent calving interval.

The delay in puberty and the consequent delay in conception is one of the important causes of low reproductive efficiency of this species, thus lengthening non-productive life. Large scale studies have focused on the use of hormones and other veterinary medicines for augmenting reproductive performance, which are considered economically not affordable to Indian farmers. Using the synthetic chemical (medicine, hormones) to treat the reproductive problem but it causes public health hazards and severe side effects on the fertility of the animals. But herbal medicine lowers side effects, more benefits and less health hazards. Herbs can play an effective role in efficient reproductive management and successful rearing of our animals (Chandra *et al*., 2017).

World Health Organization (WHO) has also recommended and promotion of Ethno veterinary practices and conservation and cultivation of medicinal plants (Dalal, 1992). Certain plants, their extractions and preparations are used in fertility regulations in traditional medicine. Asparagaceae is common at low altitudes in shade and in tropical climates throughout Asia, Australia and Africa. Out of several species of asparagus grow in India. *A. racemosus* (Shatavari), an important herbal medicinal plant of tropical and sub-tropical regions in India, has medicine importance due to presence of steroidal saponins, sapogenins and phytochemicals (Karmakar, 2012). Traditionally, Shatavari root powder are used for the treatment of productive, reproductive and udder ailment of livestock in several parts of India (Pandey *et al*., 2005; Kumar and Bharathi, 2012; Nigam and Sharma, 2010; Behara *et al*., 2013). It is becoming increasingly evident that many of herbal preparations are indeed considerable medicines value and needs for scientific validation. Since proving use of this herbal preparation, i.e. practices by the local and tribal community, these preparations got very little attention in the present system for the treatment of various reproductive problems in farm animals. So, there arises the need for other substitutes (herbals), which are considered safe and cheap locally available at the same time improve reproductive performance of dairy animals. Considering the above points, present study was undertaken to determine the effect of shatavari root powder supplementation during pre pubertal period on growth and reproductive performance of Murrah buffalo heifers.

MATERIALS AND METHODS

Asparagus racemosus root powder

Asparagus racemosus is a medicine plant. Different species of Asparagus are present in India and all over the world. Among approximately 300 species distributed around the world, 22 species of *Asparagus* have been recorded in India where, *A. racemosus* is most used as a medicinal plant in traditional medicine (Bopana and Saxena, 2007). *Asparagus racemosus* is a woody climber growing to 1 to 2 m in height. It was botanically described in 1799. Due to its multiple uses, the demand for *Asparagus racemosus* is constantly on the rise. The leaves are like pine needles, small and uniform and the flowers are white and have small spikes.

Every part of *Asparagus racemosus* as- stem, root, leaves, are used to prevent disease in both humans and dairy animals.

Animals and experimental design

The present study was conducted in the experimental cattle shed of National Dairy Research Institute Farm, Karanal, India. Eighteen Murrah buffalo heifers were selected randomly. The animals were divided into two groups (Control and Treatment) consisting of six animals in each group on the basis of their body weight and age. The mean body weight (220.05±7.11 kg) and age (18.5±0.77 months) of three groups did not differ statistically (P>0.01) at the beginning of experiment. The ethical committee for animal experiments (ECAE) of the National Dairy Research Institute approved the animal use protocol, management practices and the experimental design for the study.

Management and treatments

The heifers were housed individual pens and fed a concentrate mixture (Cured protein 20% and 70% Total digestible nutrient) and green fodders (Oats, Maize, Jowar and Berseem according to availability in the farm) every day according to standard (NRC, 2001). Fresh water was provided ad libitum daily. Using an electronic balance, all experimental animals was weighed on two consecutive days at 15-days intervals before any feed was offered. Thus, each record of body weight was an average of two observations. The feed requirement of the individual animal was determined on the basis of body weight. Dry matter intake of each group was recorded at fortnightly interval on three consecutive days during which weighed amounts of concentrate mixture green fodder and dry roughages were offered daily and residues weighed, and dry matter determined. Dry matter content of fresh as well as leftover fodder was estimated by drying the samples in electronic oven at 100±1ºC for 24 h. The fortnightly body weight of each animal was recorded consecutively for two days on a platform of electronic balance with high accuracy before providing the animals with any feeds stuff or water. Thus, each observation on body weight was on average of two observations. The heifers in Treatment group were additionally supplemented with *Asparacus racemosus* (Shatavari root powder) at the rate of 100 mg /kg Live body weight /heifer mixed with concentrate feed once in the morning during experimental period. All the heifers were given three weeks an adaptation period.

Blood sampling and analysis

The blood samples (10 ml) were collected from jugular vein into Vacutainer tubes early morning before offering any feed from all experimental animals at fortnightly interval during experimental periods. Immediately after sampling the blood centrifuged at 3000 rpm for 15 to 20 minutes and the plasma was within one hour after sampling separated and stored frozen at (-20°C) in the storage capacity of 2 ml cryovials until analyzed. Plasma progesterone was estimated by enzyme immunoassay kits.

Age at puberty and Ultrasound scanning

Puberty was confirmed by estimating peripheral level of progesterone. Plasma progesterone concentration more than 1 ng/ml in two consecutive assessments at 15 days interval was the criteria for the onset of puberty. The mid date between last sample with >1 ng/ml and first sample with >1 ng/ml was 1ng/ml was considered as date of puberty (Halder and Prakash, 2005). Ultrasound scanning examination was also carried out 15 days

interval to assess the ovarian activity including development of graffian follicles and presence of corpus luteum. The age and body weight at puberty for each animal was recorded. Ultrasound scanning was performed transrectaly by using (Alokal 500V ultrasound scanner) equipped with a 7.5 MHz linear transducer in each experimental buffalo heifers at 15 days interval and on the day of oestrus to examine the presence of corpus luteum and ovarian follicular characteristics, respectively. Based on the diameter the follicles were counted and classified small size (<3 mm), medium size (3 to 7 mm) and large size (>7 mm).

Breeding management

The heifers were visually observed for estrus detection twice daily (6.00 AM and 6.00 PM) by herd-man. They were observed for bellowing, vulvar tumefaction, frequent urination and vaginal mucous discharge (Perera, 1999). Also, a standing of heifers to be mounted by the vasectomised bull maintained with them was the most reliable sign for estrus detection. The heifers were artificially inseminated using proven fertile frozen semen on the basis of AM/PM system i.e. heifers detected in estrus at the evening were inseminated at morning and vice versa. The age and body weight at first insemination for each animal was recorded. The inseminated heifers were observed for estrous return on day 19 to 23 post breeding. The non-return heifers were examined by transrectal ultrasound scanning of the uterus on day 40 post breeding. The conception rate and number of services per conception were also recorded. The age and body weight at first conception for each animal was recorded.

Statistical analysis

The experimental data were analysed by ANOVA for completely randomised design using SAS version 7.0 programmes using the linear equation model with interaction. The differences in the two parameters among the comparable groups were analysed

RESULTS AND DISCUSSIONS

Dry matter intake (DMI)

The overall fortnightly mean ± S.E. of dry matter intake (DMI)/100 kg body weight were found to be 2.55±0.01 and 2.64±0.01, in Control and Treatment group, respectively. However, DMI as a percent of live body weight showed statistically significant (P>0.05) among the Treatment and Control groups (Figure 1). Shatavari contains tannin which acts as bypass protein. Tannin binds with available protein in the rumen converted in Rumen Degradable Protein (RDP) to Rumen Undegradable Protein (RUP) which absorbs intestines. Shatavari also contains saponin (glycoside amphipathic compound) which acts as surfactant which changes the permeability of gut membrane and increases the absorption capacity of gut and increases the DMI of animal (Jamra, 2012). In the present experiment, increased in DMI during pre pubertal period in Shatavari supplemented group could be attributed to anabolic effect of *A. racemosus (*Sharma *et al*., 1986; Panigrahi *et al*., 2005; Kumar, 2014). Feeding herbal formulation containing 25% Shatavari significantly enhances dry matter intake (DMI) by 10.97% in buffaloes (Mahantra *et al*., 2003) and in cows at the rate of 100 g on alternate day (Berhane *et al*., 2002).

Average body weight gain

The total difference (starting to end of experiment) in body weight gain of the Murrah

heifers averaged were 177.03 and 203.0 kg for Control and Treatment group, respectively. The body weight gain Treatment group in the present investigation was significantly (P<0.01) higher than the animals of Control groups (Figure 2). In the present study the body weight gain in shatavari Supplemented group was significantly (P<0.01) higher than the animals of Control groups. The result of *A. racemosus* supplementation and its effects in influencing the growth performance is in agreement with earlier studies that have demonstrated a positive impact on growth promoting effect can be ascribed to its adaptogenic property of *A. racemosus* (Sharma *et al*., 1986) also reported that the supplementation of *A. racemosus* has been found to promote digestibility and dry matter intake in healthy as well as in problematic animals without disturbing rumen protozoan(Sharma *et al*., 1986) further strengthening present study findings. The overall mean± S.E of average daily weight gain (ADWG) g/day were 485.66±22.09 and 516.02±21.61 for control and treatment groups, respectively. The average daily weight gain of animals Treatment group was statistically (P<0.05) higher than untreated Control group. The growth promoting effect was indicative of its anabolic effect and ascribed to its adaptogenic substances (Sharma *et al*., 1986). These findings further support our study.

Reproductive parameters

Age and weight at puberty

The overall least squares Mean±S.E. of age and body weight at puberty in Control and Treatment group of Murrah heifers were 821.66±15.38 (Figure 3) and 764.00±12.66 (Figure 4) days for Control and Treatment groups, respectively and 321.55±15.96 and 337.96±17.81 Kg for Control and Treatment group, respectively. The mean body weight did not differ significantly between Control and Treatment group. The difference in age at puberty between Control and Treatment groups was found to be significant (P<0.05). It also contains Shatavarins, the steroidal saponins, may be responsible for the hormonal like effect of shatavari and explain its traditional use as a reproductive tonic (Chang and Liao, 1987). The results of early puberty in shatavari supplemented buffalo heifers due to presence of steroidal saponins. Asparagus roots contain flavonoids, which are groups of hormones like diphenolic phytoestrogens. These Phytoestrogens are group of plant derived compounds that structurally and functionally mimic the actions of mammalian estrogen. More specifically, phytoestrogens mimic the action of 17β-estradiol and bind weakly to estrogen receptor and induce production of sex hormone binding globulin (SHBG) in the liver and in this way influence sexual hormone metabolism. Natural or synthetic estrogens have serious consequences on the reproductive cycle in humans and animals. Precocious vaginal opening, which is an indicator of attainment of puberty, could occur in response to actions of estrogens or estrogen-like substances (Marty *et al*., 1999).

Heifers that grow faster, show short prepubertal period and calve at younger age, have greater lifetime productive efficiency with higher fecundity than those heifers exhibiting slow growth rate, long prepubertal period and calving at older age (Berhane and Singh, 2002). Shatavari, a general tonic and a female reproductive tonic, contains 9, 10-dihydrophenanthrene, which has been shown to interact with androgen receptors and may therefore inhibit androgen-dependent prostatic growth (Short and Bellows, 1971).

Age and weight at sexual maturity

The average age at sexual maturity (ASM) was 876.33±16.41, and 831.16±10.24 days for Control and Treatment group, respectively. Whereas the difference in age at sexual maturity among between Control and Treatment group was found be significant (P<0.05). The mean average body weight at sexual maturity was 354.75±18.44 and 375.35±16.55 kg for Control and Treatment group, respectively. However, body weight difference was not statistically significant among the groups. But higher body weight was attained in Treatment group. Age at sexual maturity is a function of body weight rather than age. It is also observed in the preset study. Early maturity in his considered as a character of great economic importance as from birth to date of calving, the animal produces nothing except dung and urine, the value of which is negligible as compared to the cost of maintenance. Nanda *et al*. (2003) observed that better nutrition reduces the age of maturity in buffalo heifers. Shatavari (*Asparagus racemosus*) can be used as a feed supplement for growth and puberty in dairy animals. It has anti-stress properties (Kumar *et al.,* 2008) and causes early puberty and increase in weight of ovaries, uterus and teats in female (Sharma, 2011) Feeding of roots of *Asparagus racemosus* (shatavari) and Phoenix acaulis Roxb. (Dwarf date Palm) to Sahiwal heifers lower the age at puberty ,age at first service(Jamra, 2012).

The average age at first conception was 979.33±15.35 and 917.5±13.52 days for Control and Treatment group, respectively. The difference in age at age at first conception among the group was found be significant (P<0.05). The heifers attain early puberty, consequently early conception and results in high reproductive efficiency and lengthening of the productive life. Similar trend was observed in the present study. This amazing herb is not fully explored scientifically in dairy animals to improve reproduction. However, it has been reported that supplementation of *Asparagus racemosus* (100 g at alternate day) led 100% estrus and 75% conception in crossbred cattle within 90 days of calving (Berhane, 2000).

Plasma progesterone concentration

Plasma progesterone (P4) concentration (ng/ml) levels were estimated in blood plasma samples of Murrah buffalo heifers collected at fortnight interval (Figure 3 and Figure 4). The plasma progesterone levels at the beginning of the experiment remained similar both control and treatment groups (P>0.05). Progesterone concentrations were remaining less than 1.00 ng/ml during pre-pubertal period of Murrah buffalo heifers control as well as in the Treatment group (ranging from 0.1 to 0.3 ng/ml). However, a greater number of heifers's attained early puberty in Treatment group and required a smaller number of Artificial inseminations for conception as compared to Control group (Table 1).

Ovarian follicular characteristics

Ovarian follicular characteristics on the day of estrus in Murrah buffaloe heifers in Control and Treatment groups are presented in (Table 2). The number of small size (<3 mm) and medium size follicles (3 to 6 mm) differ significantly (P<0.05) between Treatment and Control groups. However, there was no significant number of large size follicle (P>0.05) among Treatment and Control groups. But total number of follicle and diameter of large follicle differ significantly (P<0.05). The number of small and medium size follicle was more in Treatment groups due to steroidogenic activity of *Asparagus racemosus* (Shatavari). Jayakumar

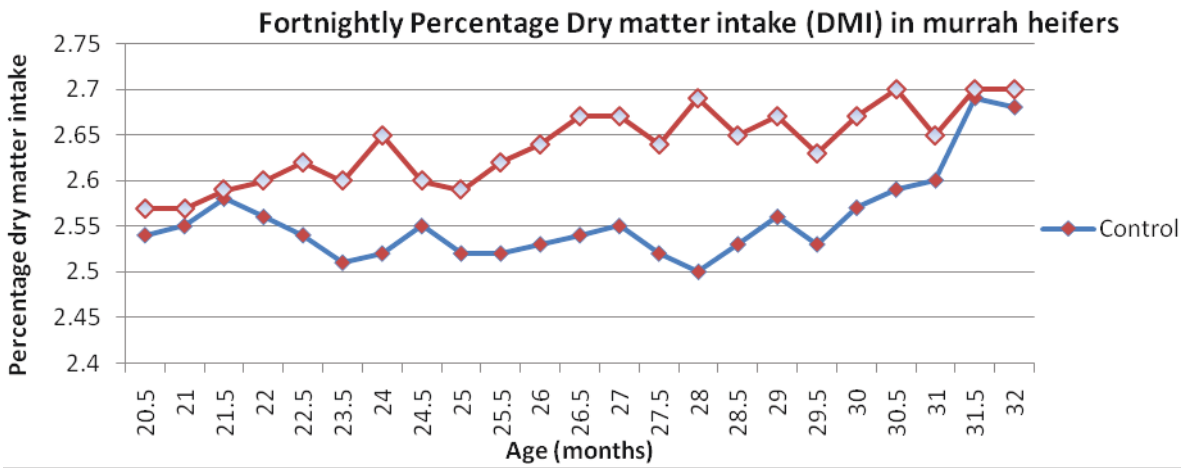

Figure 1. Fortnightly percentage dry matter intake (DMI) in Murrah buffao heifers.

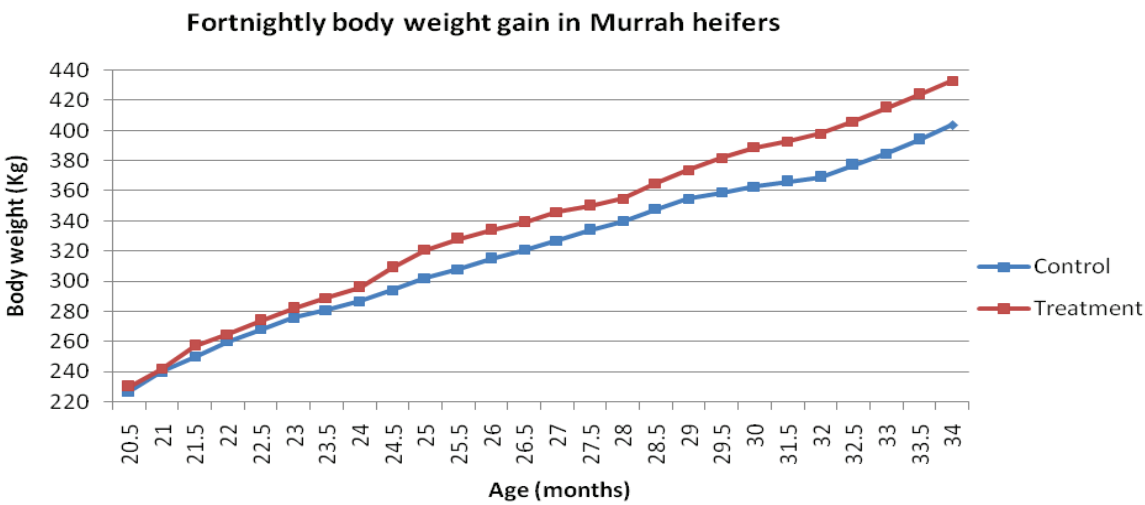

Figure 2. Fortnightly body weight gain (kg) in Murrah buffalo heifers.

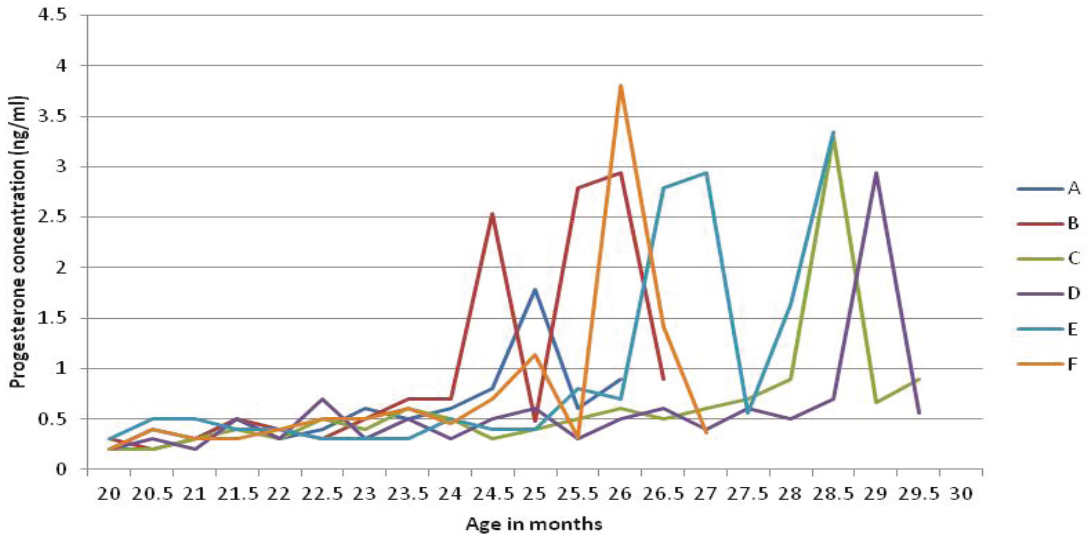

Figure 3. Blood progesterone concentrations in buffalo heifers in Control group.

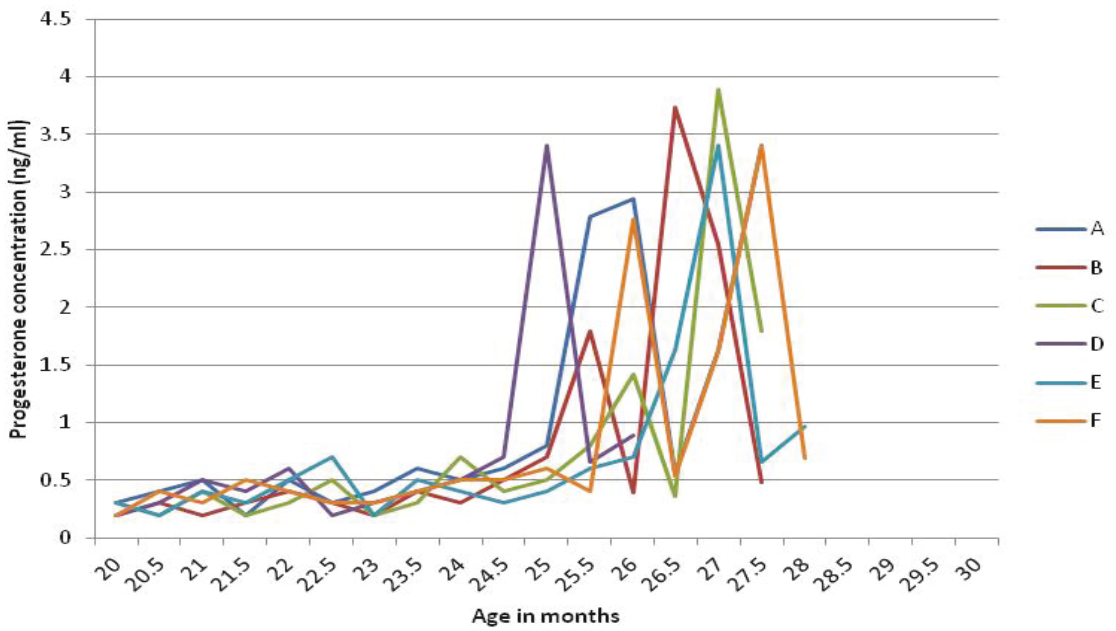

Figure 4. Blood progesterone concentrations in buffalo heifers in treatment (Shatavari root powder supplemented) group.

Table 1. The effect of Shatavari supplementation on follicular characteristics of Murrah buffalo heifers.

Parameter	Control group	Treatment group
Number of animal attain puberty	6	6
Number of animal inseminated	6	6
Number of animal pregnant at 1st AI	2	4
Number of animal pregnant at 3rd AI	4	2
Conception rate (%) at 1st AI	35	65
Conception rate (%) at 3rd AI	65	35

*Means bearing different superscript [a] and [b] differ in a row (P<0.05).

Table 2. The effect of Shatavari supplementation on conception rate and follicular characteristics of Murrah buffalo heifers.

Parameters	Control group	Treatment group
Number of small follicles (<3mm)	$1.6^{a}\pm0.14$	$2.4^{b}\pm0.3$
Number of medium follicle (3-6 mm)	$1.2^{a}\pm0.2$	$1.8^{b}\pm0.21$
Number of Large follicle (> 6 mm)	1.0±0.00	1.3±0.12
Total number of follicles	$3.7^{a}\pm0.42$	$5.6^{b}\pm0.66$
Diameter of large follicle	$8.3^{a}\pm0.25$	$9.7^{b}\pm0.50$

*Means bearing different superscript [a] and [b] differ in a row (P<0.05).

S. (1997) reported that supplementation of *Aloe vera* at a dose level of 300 and 500 mg/kg body weight had improved the follicular development and steroidogenic activity. Supplentation of Murrya koenigii (curry leaf) significantly advance the onset of puberty and increased the number of large surface and embedded follicles and increase expression ovarian glucose -6 and 3 beta HSD enzymes function in cattle (Kabir *et al*., 2001).

CONCLUSION

In conclusion, Supplementation of shatavari root powder, during pre pubertal period significantly improves body weight and attains early age at puberty, in Murrah buffaloe heifers.

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