SUPPLEMENTATION OF FERMENTED YEAST CULTURE AUGMENTS THE GROWTH AND REDUCES THE AGE AT PUBERTY IN MALE MURRAH BUFFALO CALVES

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ABSTRACT

The present study aims to investigate the effects of supplementation of fermented yeast culture (FYC) on growth and age at attainment of puberty in male Murrah buffalo calves. Twelve buffalo calves of 10 months of age were categorized into supplemented (n = 6) and control (n = 6) groups. The experiment was conducted during10–14 months of age of the calves (growing period) and later form 18 months to till their attainment of puberty (peripubertal period). The supplemented group was offered with FYC 12 g/animal/day during growing period and 24 g/animal/day during peipubertal period. Dry matter intake (DMI), BW, feed conversion efficiency (FCE), and feed conversion ratio (FCR) were recorded at regular intervals. Blood samples were collected at weekly intervals, and hormone concentrations such as insulin–like growth factor–1 (IGF–1), haptoglobin (Hp), and testosterone were estimated. The plasma IGF–1 concentrations were significantly ($P < 0.05$) higher in the supplemented group during growing period as compared to the control. The overall mean ± SEM plasma testosterone concentration in supplemented group was significantly higher ($P < 0.05$) than the control group. Throughout the course of study, BW, average daily gain, and feed conversion efficiency were significantly ($P < 0.05$) higher in the supplemented group. The supplemented Murrah bulls attained puberty by 25 months of age, whereas the control animals attained puberty by 27 months of age. This study indicated that the supplementation of FYC augments the growth and reduces the age at puberty in male Murrah buffalo calves.

Keywords: average daily gain, insulin–like growth factor–1, male calves, puberty, fermented yeast culture

INTRODUCTION

Buffaloes are the major source of milk production, and they contribute significantly to the economy of many countries in Southeast Asia. However, the male buffaloes are known for their delayed puberty and sexual maturity. On an average, the male Murrah buffaloes attain puberty between 24 and 30 months of age. The mean age at puberty in case of male Murrah buffaloes as observed by Kotayya and Narasimaharao (1972) was 26.7 ± 1.48 months. Gulia *et al*. (2010) observed that the testosterone levels were comparatively lower in buffalo bulls as compared to the Sahiwal and Karan Fries (KF) bulls.

To yield maximum production efficiency

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by ruminants, many of the researchers (Fallon and Harte, 1987; Wagner *et al*., 1990; Mutsvangwa *et al*., 1992) manipulated the ruminal microbial ecosystem by addition of yeast cultures to their diet. Since, contradictory results were obtained with respect to the increase in dry matter intake (DMI) and body weight gain (BWG) with supplementation, Yoon and Stern (1995) reasoned that the dietary composition and environmental conditions also influence the growth response of animals to yeast culture. Erasmus *et al*. (1992) found that the inclusion of fungal additives in the starter resulted in increased apparent total tract digestibility, higher average daily gain (ADG), and higher total tract digestibility of fibre. Addition of Saccharomyces *cerevisiae* (SC) in a dairy calf starter significantly enhanced the DMI and growth of Holstein Friesian calves (Lesmeister *et al*., 2004). Bitencourt *et al*. (2011) observed that the improvement in digestive efficiency of ruminants by yeast supplementation depends on the yeast strain and the diet composition. Supplementation of fermented yeast culture (FYC) to the KF calves resulted in an increase in their BWG (Anand Laxmi *et al*., 2012).

Onset of puberty in cattle is under the close relationship between IGF–1and sex steroids (Renaville *et al*., 1993), which in turn can modulate GnRH axis. Davis and Simmen (2006) observed that the insulin–like growth factor–1 (IGF–1) concentration was positively correlated with the growth traits. Recent studies have demonstrated that IGF–1 levels and productivity can be modulated by supplementation of FYC (Abdalla *et al*., 2013). There is an important link between feed intake, metabolic factors, and gonadotropic axis, which regulates the reproductive functions in farm animals (Velazquez *et al*., 2008; Deb *et al*., 2014). Elevation of serum haptoglobin (Hp) levels is generally non–specific concerning the nature of the inciting agent, and therefore can be used to gauge the extent and activity of many different pathological processes (Eurell *et al*.,1992). The present study was designed to investigate the relation of plasma IGF–1 with body weight and feed parameters, and to evaluate the effect of supplementation of FYC on productive performance and age at attainment of puberty of male Murrah buffalo calves.

MATERIALS AND METHODS

Selection of bull calves and experimental schedule

The experiment was conducted at the National Dairy Research Institute (NDRI), Karnal, Haryana, India. Twelve male Murrah buffalo calves of 10 months of age, with low mean ± SE body weight (BW) of 110 ± 10 kg, were selected for the experiment. The calves were divided into two groups, supplemented (n = 6) and control (n = 6). The experiment was carried out during 10–14 months of age of the calves (growing period) and later form 18 months to till they attainment puberty (peripubertal period).

Feeding schedule, blood sampling, and hormone assays

Feed was offered to all the calves as per NRC (2001) recommendations. Wheat straw and concentrate were offered in the ratio of 1:1 3 kg/100 kg BW/animal. Additionally, green roughage was also offered 2.5 kg/calf/day. As the body weight of calves increased, the feed was offered according to their requirement based on body weight. Along with the normal diet, the supplemented group was offered with FYC (*Saccharomyces cerevisiae*) 12 g/animal/day from 10–14 months of age. The

supplementation of FYC was withdrawn from 14–17 months of age. From 18 months of age, FYC was supplemented 24 g/animal/day until the attainment of puberty. The colony–forming unit of *Saccharomyces cerevisiae* was 1.5×10^7/g. Throughout the course of the study, all the animals had free access to drinking water. Blood samples were collected from all the animals at weekly intervals during the experimental period, by jugular vein puncture. Immediately after collection, the blood samples were centrifuged at $1077 \times g$ for 15 min, and the plasma samples were stored at −20°C until they were analyzed for hormones. Insulin–like growth factor–1 and Hp concentrations were estimated from all the samples collected during the experimental period, whereas testosterone was estimated only from the samples collected after 18 months of age of the animals. Concentrations of plasma IGF–1, Hp, and testosterone were estimated by commercially available enzyme immunoassay kits (Biotech Inc. Co. USA.). The intra– and inter–assay coefficients of variation were less than 10% for all the hormones.

Growth and feed related traits

Daily feed intake and feed refusal were recorded. Dry matter intake was calculated as the difference between feed intake and refusal. Feed conversion ratio was computed as the ratio of DMI to ADG while the inverse of this ratio was the feed conversion efficiency. Body weight of the animals was recorded at monthly intervals.

Scrotal circumference, sex drive, and semen analysis

Scrotal circumference was measured according to the method given by Chenoweth and Ball (1980). All the animals were subjected to mounting from 22 months of age and the semen samples were collected by using artificial vagina. Semen analysis included the recording of ejaculate volume, assessment of viability, and enumeration of sperms. Sex drive of each bull was assessed by recording the reaction time for the ejaculatory thrust to take place (El-Azab *et al*.,1980). Approval from institutional ethics committee was obtained to perform the experiment.

Statistical analysis

For all the parameters, the mean ± SE value obtained at a particular age in the supplemented group was compared with its respective value of the control group by student's t–test. The student's t–test was also used to compare the overall mean ± SEM values of the supplemented group with that of the control group during growing and peripubertal periods, for all the parameters. Graphpad prism (version 5) software was used for statistical analysis.

RESULTS

The mean ± SE plasma IGF–1 concentrations at the beginning of the experiment in control and supplemented groups were 42.89 ± 0.58 and 42.66 ± 0.84 ng/mL, respectively. The IGF–1 concentrations in both the groups increased with the increase in age of the animals (Fig. 1a). The IGF–1 concentrations observed between 10–14 months of age in the supplemented group were significantly ($P < 0.05$) higher than the control (Fig 1a). The concentration of plasma testosterone increased significantly ($P < 0.05$) in the supplemented group between 20–23 months of age (Fig. 1c), and the overall mean value was also significantly greater ($P < 0.05$) when compared

with the control group (Table 2). The concentration of plasma Hp fluctuated between 400–750 ng/mL in both the groups, the difference between the values was not significant at any time interval (Fig. 1b).

The estimated mean ± SE body weight of calves between 11–14 and 19–23 months of age were significantly ($P < 0.05$) higher for the supplemented group than the control group (Fig. 2a). The mean ± SE BWG and ADG values of both the groups observed during the experimental period are represented in Figure 2b and 2c, respectively. During the growing period of calves, the ADG in body weight of supplemented animals was ≥ 700g /day whereas in control group, the ADG was < 600g/day. During the peripubertal period, the average ADG in supplemented and control groups was > 500 and ≤ 500 g/day, respectively. The overall mean ± SEM BWG in supplemented group of both the growing and peripubertal animals were significantly ($P < 0.05$) higher than that of their respective control groups. The overall mean ± SEM ADG in supplemented group of both the growing and peripubertal animals were significantly ($P < 0.05$) higher than that of their respective control groups. The average DMI/animal/day/100 kg body weight in supplemented and control groups was 2.55 and 2.53 kg, respectively and the difference was non–significant between the groups. Feed conversion ratio (FCR) was comparatively higher in the control group, whereas FCE was higher in the supplemented group (Table 1). The mean ± SEM FCR and FCE parameters were significantly higher ($P < 0.05$) in the control and supplemented groups, respectively (Table 2). For the control group, the study was extended until 28 months of age; the mean ± SEM values for different parameters of the control group during their peripubertal period (24–28 months) are given in Table 3.

The average scrotal circumference at 23 months of age in control and supplemented groups was 23.4 and 25.5 cm, respectively. Sperm concentration of ≥ 50 million/mL and sperms with > 10% progressive motility was observed in the ejaculates of all the animals of supplemented group at 25 months of age. Whereas in the control group, only 4 out of 6 animal's ejaculates had a sperm concentration of ≥ 50 million/mL and sperms with > 10% of progressive motility. The differences in the sperm concentration, volume, motility, and viability between the supplemented and control groups were non–significant ($P > 0.05$).

DISCUSSION

In the present study, 12 low body weight Murrah buffalo calves of 10 months of age, were categorized into supplemented (n = 6) and control (n = 6) groups. The supplemented group was provided with FYC 12 g/animal/day during growing period and 24 g/animal/day during peripubertal period, in order to determine its effect on growth and age at attainment of puberty.

The significant ($P < 0.05$) increase in BW, BWG and ADG of the supplemented group observed in the present study was in accordance with the results obtained by Lesmeister *et al*. (2004) and Anand Laxmi *et al*. (2012). Along with the increased BW, Abdalla *et al*. (2013) observed a significant increase in blood glucose levels, total protein, and albumin with FYC supplementation. In the present study, DMI in the supplemented group was comparatively higher than the control, but was non-significant ($P > 0.05$). Kumar *et al*. (2011) observed similar non–significant increase in DMI with FYC supplementation. The higher FCE in the supplemented group as observed in the

present study suggests that the FYC might have improved the digestive efficiency and increased the concentration of metabolites in rumen (Kumar *et al*., 2011), which in turn might have positively affected the BW, BWG, and ADG.

As the growth rate, BW, and BWG are positively correlated with the plasma IGF–1 concentrations (Groenewegen *et al*., 1990; Davis *et al*., 1995; Carroll *et al*., 1998), the significant ($P < 0.05$) increase in these growth parameters observed in this study justifies the higher IGF–1 levels of the supplemented group. Badinga *et al*. (1991) also reported the increased concentration of plasma IGF–1 in well–fed pubertal cattle when compared with the control. The plane of nutrition has a significant impact on the circulatory levels of IGF–1 (Brito *et al*., 2007). The FYC itself is a rich source of soluble growth factors such as amino acids, vitamins and organic acids (Newbold *et al*., 1996; Callaway and Martin, 1997). A few reports (Stubbs *et al*., 2002; Zhao and Sun, 2010) indicated that volatile fatty acids and certain amino acids could modulate the release of IGF–1 i*n vivo* and i*n vitro*.

In the present study, the plasma testosterone levels increased significantly ($P < 0.05$) in the supplemented group during 20–23 months. The average scrotal circumference observed at 24 months of age was also higher in the supplemented group. All the animals in the supplemented group attained puberty as early as 25 months of age, whereas only 4 out of 6 animals of the control group attained puberty by 27 months of age. Thomas *et al*. (2002) reported a positive correlation

Table 1. Feed conversion ratio and feed conversion efficiency in the supplemented and control groups, during growing (10-14 months) and peripubertal (18-23 months) periods of male Murrah buffaloes.

Age in Months	FCR		FCE	
	Control	Supplemented	Control	Supplemented
10	11.60 ± 1.02	9.40 ± 0.98	8.50 ± 0.65	10.56 ± 0.99
11	15.05 ± 1.14*	10.10 ± 0.89	6.64 ± 0.52	12.20 ± 0.98*
12	10.49 ± 1.03	8.36 ± 0.98	9.52 ± 0.53	15.50 ± 0.92*
13	14.25 ± 1.12*	10.30 ± 1.10	7.01 ± 0.67	11.84 ± 0.58*
14	12.50 ± 1.02*	9.30 ± 0.93	7.94 ± 0.67	10.67 ± 0.63*
18	7.78 ± 0.5	6.73 ± 0.39	15.72 ± 0.56	17.42 ± 0.42
19	6.68 ± 0.52*	5.57 ± 0.34	16.60 ± 0.58	18.57 ± 0.28*
20	7.21 ± 0.40	6.43 ± 0.46	12.24 ± 0.59	16.43 ± 0.32*
21	7.06 ± 0.4	6.82 ± 0.46	13.24 ± 0.41	15.52 ± 0.39
22	7.20 ± 0.42	6.72 ± 0.45	11.19 ± 0.48	14.46 ± 0.39*
23	7.70 ± 0.42*	5.96 ± 0.42	12.87 ± 0.51	16.45 ± 0.30*

FCR- Feed conversion ratio, FCE-Feed conversion efficiency. Values are represented as Mean ± SE, *$P < 0.05$.

Table 2. Variations in the overall mean ± SEM of different parameters in the supplemented and control groups, during growing (A; 10-14 months) and peripubertal (B; 18-23 months) periods of male Murrah buffaloes.

A	Control	Supplemented
IGF-1 (ng/mL)	49.29 ± 1.32	55.88 ± 1.20
Hp (ng/mL)	505.6 ± 30.07	427.77 ± 30.21
BW (kg)	265.8 ± 9.23	315.20 ± 15.63*
BWG (kg)	17.30 ± 1.58	25.48 ± 2.43*
ADG (kg)	0.56 ± 0.05	0.83 ± 0.08*
FCR	12.78 ± 0.83*	8.89 ± 0.81
FCE	7.92 ± 0.51	11.55 ± 1.12*
B		
IGF-1 (ng/mL)	115.4 ± 9.2	132.2 ± 9.6
Hp (ng/mL)	632.7 ± 21.32	644.8 ± 19.8
BW (kg)	345.5±9.63	352.7±10.52
BWG (kg)	12.33 ± 1.33	21.00 ± 1.03*
ADG (kg)	0.44 ± 0.03	0.75 ± 0.04*
Testosterone (ng/mL)	0.58 ± 0.10	1.56 ± 0.44*
FCR	7.27 ± 0.17	6.70 ± 0.35
FCE	13.31 ± 0.85	17.53 ± 0.69*

IGF I- Insulin like growth factor I, Hp- Haptoglobin, BW- Body weight, BWG- Body weight gain, ADG- Average Daily gain, FCR-Feed conversion ratio, FCE-Feed conversion efficiency.*P<0.05.

Table 3. Mean ±SE values of different parameters in the control group during 24-28 months of age.

Age in Months	IGF I (ng/mL)	HP (ng/mL)	BW (kg)	BWG (kg)	ADG (kg)	Testosterone (ng/mL)
24	145.23 ± 3.23	615.25 ± 6.35	296 ± 5.23	12 ± 0.25	0.4 ± 0.02	1.99 ± 0.05
25	150.65 ± 2.25	650.15 ± 7.12	303 ± 4.06	12 ± 0.42	0.4 ± 0.03	2.08 ± 0.06
26	155.45 ± 4.36	689.78 ± 6.27	315 ± 4.21	11 ± 0.28	0.37 ± 0.02	3.15 ± 0.14
27	160.36 ± 3.24	612.36 ± 5.69	325 ± 3.25	10 ± 0.53	0.33 ± 0.01	3.23 ± 0.14
28	165.39 ± 2.69	545.89 ± 5.02	337 ± 3.42	12 ± 0.45	0.4 ± 0.01	3.48 ± 0.16
Mean±SEM	**155.4 ± 2.89**	**622.7 ± 19.42**	**315.2± 6.08**	**11.3± 0.33**	**0.38 ± 0.13**	**2.48 ± 0.68**

IGF I- Insulin like growth factor I, Hp- Haptoglobin, BW- Body weight, BWG- Body weight gain, ADG- Average Daily gain.

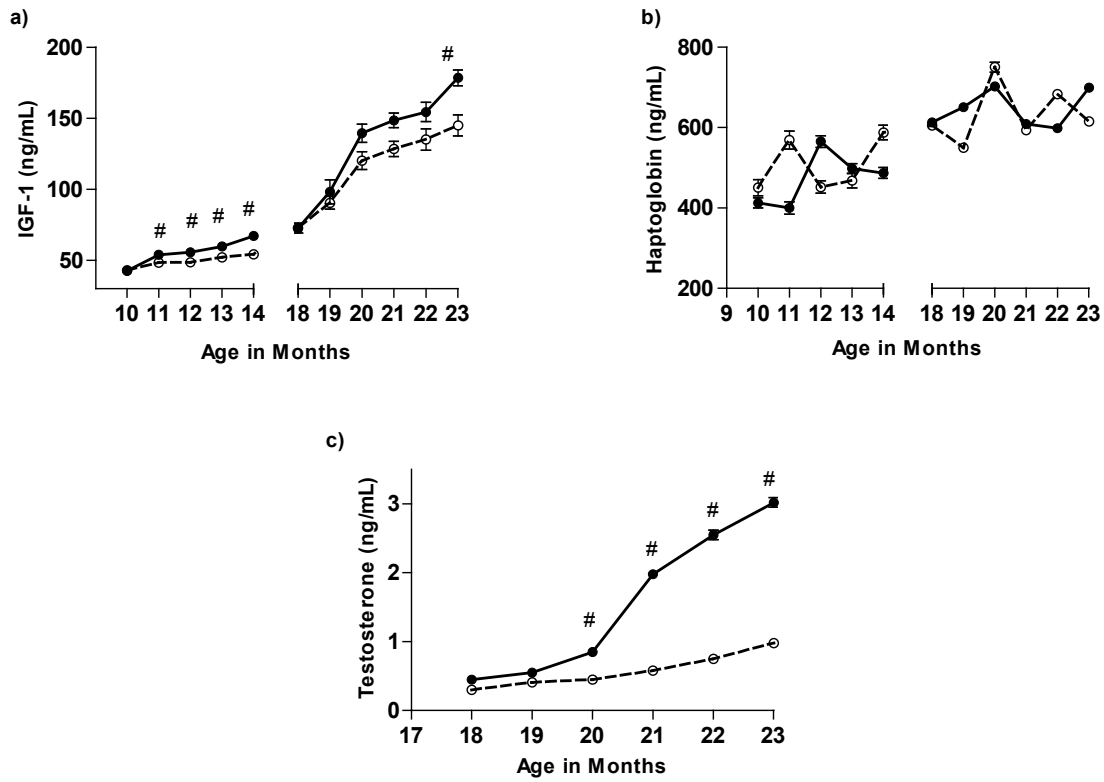

Figure 1. Variations in the mean (± SE) IGF-1, haptoglobin and testosterone concentrations in the supplemented (⟶) and control (⟶) groups, during growing (10-14) and peripubertal (18-23) periods of male Murrah buffaloes. Significant difference between means is denoted by $^{\#}(P < 0.05)$.

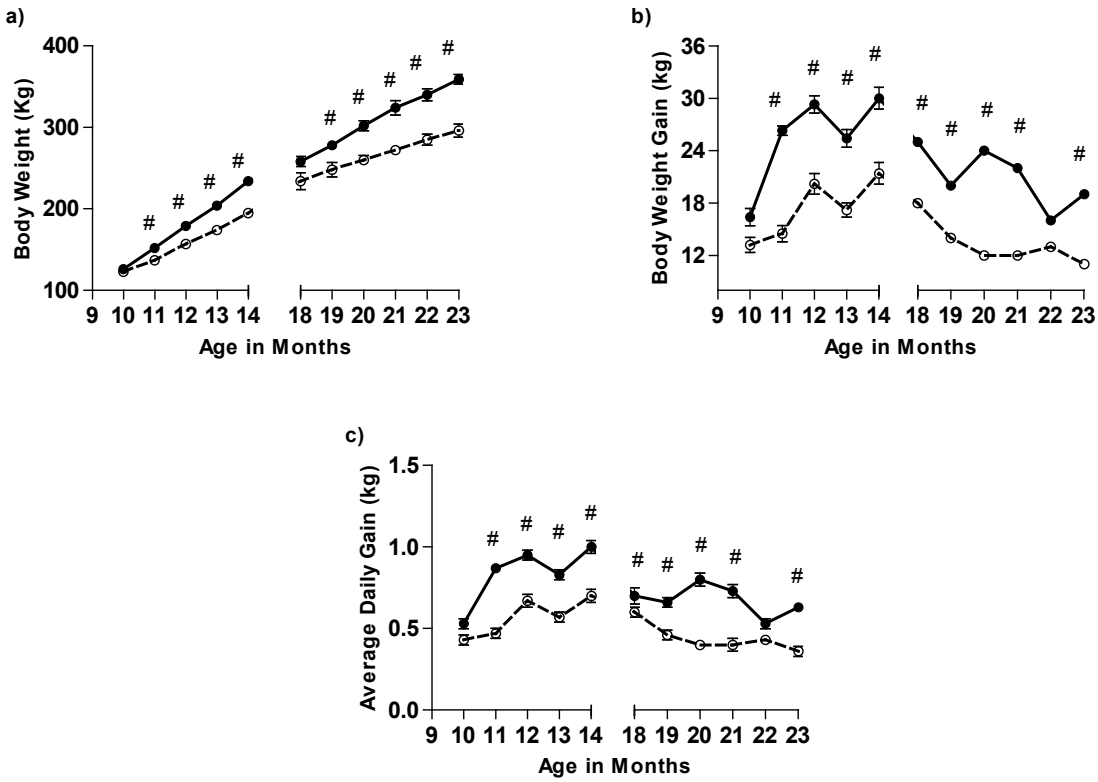

Figure 2. Variations in the mean (± SE) body weight, body weight gain, average daily gain and dry matter intake in the supplemented (⊸) and control (⊸) groups, during growing (10-14) and peripubertal (18-23) periods of male Murrah buffaloes. Significant difference between means is denoted by $^{\#}(P < 0.05)$.

between serum IGF–1 levels and reproductive parameters in Angus bulls. Insulin–like growth factor–1 can cross the blood brain barrier and enter the central nervous system (Pan and Kastin, 2000). Studies conducted on mice, indicated the IGF–1 as a neurotrophic factor that could regulate the gonadotropin releasing hormone (GnRH) system, and stimulate its expression (Daftary and Gore, 2003). This may be the one of the mechanisms, whereby the increased concentration of circulatory IGF–1 might have advanced the age at puberty in the supplemented group of this study. Gonadotropins, testosterone, IGF–1, and other factors, such as cytokines, activin, inhibin, and follistatin regulate the sperm production (Madhukar and Rajender, 2009; Magon *et al*., 2011). Brito, (2006) suggested the IGF–1 as a possible factor which transforms body nutritional status to the GnRH pulse generator. From the present study, it was observed that the age at puberty was positively associated with the BW, BWG, ADG, and plasma concentrations of IGF–1 and testosterone, and could be manipulated by simple biotechnological tool like supplementation of FYC. Therefore, it was concluded that the supplementation of FYC augments the growth and reduces the age at puberty in male Murrah buffalo calves.

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