RESEARCH ARTICLE

Effect of High Plane of Nutrition on Blood Biochemical Profile and Onset of Puberty in Prepubertal HF X Kankrej Crossbred Heifers

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Abstract

This study was initiated on 24 prepubertal Holstein x Kankrej crossbred heifers of nearly identical age (7-9 months) and body weight (130–140 kg) at University farm to evaluate the effect of high plane of nutrition on blood biochemical and minerals profile and the age at puberty. Twelve heifers were managed under routine farm feeding (control) and the rest 12 under ideal optimum feeding regime (treatment) that included extra 1 kg concentrate, 30 g min mix and *ad-lib* dry fodder. The body weight and ovarian ultrasonography together with blood sampling was carried out at monthly interval from 10–18 months of age to study the ovarian dynamics and blood biochemical changes. High plane of nutrition to growing heifers was beneficial in reducing the age of onset of puberty (by 2–3 months) compared to routine farm fed group. The mean plasma total protein and cholesterol concentrations showed a rising trend with significant variations from 10 to 16 months of age, where it got mostly stabilized indicating adult profile. The activity of enzymes GOT and GPT also rose gradually and significantly from 10 months till 14–15 months of age, and thereafter it remained more or less static till 18 months of age. The levels of both these enzymes were higher, with lower protein and cholesterol, in control than the treatment group from 15–16 months of age onwards. The mean plasma levels of both calcium and phosphorus increased gradually and significantly with advancing age till 16–17 months of age, with little higher values in supplemented than a control group. The plasma levels of zinc, iron, copper, and cobalt also showed rising trend with significant differences between 10^{th} and 12^{th}–14^{th} months of age, and from 15^{th}–18^{th} months of age the levels were statistically the same in all the groups with slightly higher values in the treatment group.

Keywords: Age at puberty, Blood biochemical profile, Crossbred heifers, Plane of nutrition.

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Introduction

Early attainment of puberty and reduced age at first calving are economically important in dairy animals. Pubertal development involves physical, physiological, and behavioral changes that are linked to the hypothalamo-pituitary-gonadal (HPG) axis (Sisk and Foster, 2004), and the genetic and nutrition has a significant influence on it. Signals mediating nutritional and metabolic information are mainly perceived at the level of the hypothalamus that controls various neuroendocrine functions, including puberty (Schneider, 2004). The average daily body weight gain in HFxK (inter-se) female calves is around 450 g under ideal feeding and management conditions attaining the age of puberty at around 16–18 months and age at first calving (AFC) of 30-32 months (Anonymous, 2018). However, under field conditions, pubertal age is delayed up to 30 and 36 months, mainly for malnutrition. The nutritional intervention has been shown to enhance puberty and AFC in dairy heifers (Day and Nogueira, 2013; Gupta *et al.,* 2016). It has also been shown to accelerate the growth rate, ovarian dynamics, and endocrine profile congenial for the onset of early puberty and conception (Roberts *et al.,* 2009; Pinheiro *et al.,* 2010; Dhami *et al.*, 2019). However, the literature on the effect of such nutrition on blood biochemical and mineral profile is meager. Hence, this experiment was initiated with the hypothesis that the special nutritional care of weaned HF x K heifers would help to gain nutritional status optimum to exhibit the first estrus and conceive by 20-22 months of age.

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Materials and methods

The study was undertaken on crossbred (HF x Kankrej) heifers (n = 24) around 7-9 months of age and nearly identical body weight (130–140 kg) at Livestock Research Station, Anand Agricultural University, Anand after approval of experiment by the Institutional Animal Ethics Committee. The heifers were randomly divided into two equal groups, one half of them (n = 12) were kept under routine farm feeding schedule (control group) and rests 12 were managed under ideal optimum feeding practices (treatment group) that included extra 1 kg concentrate (22% CP), 30 g min mix and *ad lib* dry fodder. Animals of both the groups were monitored for ovarian dynamics and signs of first estrus/puberty, sexual maturity and conception rate including evaluation of ovarian dynamics by monthly per rectal palpation and ultrasound (using 5.0–7.5 MHz frequency probe, M-5 Vet, Mindray) from 10–18 months of age, along with blood sampling for biochemical and minerals profile.

Blood plasma samples with a drop of merthiolate (0.1% as preservative) were stored in a deep freeze at -20°C until analyzed. The levels of plasma total protein, total cholesterol, calcium, phosphorus, and magnesium were estimated using assay kits and procedures of Crest Biosystems, Goa, India, on biochemistry analyzer (Nova 2021, Analytical Technol. Pvt. Ltd., Vadodara). The concentrations of plasma trace minerals were determined in wet digested samples on ICP-OES (Optical Emission Spectrometer; Model Optima 7000 DV; Perkin-Elmer, USA). The data on biochemical profiles were statistically analyzed using ANOVA, DMRT, and t-test employing SPSS software version 20.0 (Snedecor and Cochran 1994).

Result and discussion

Plasma Biochemical and Enzymatic Profiles

The mean plasma total protein and total cholesterol concentrations showed a rising trend with significant variations throughout study from 10 months till 16 months of age, from where it did not vary significantly till 18 months of age (Table 1). Serum proteins constitute a portion of the amino acid pool in the body. The concentration of total protein in blood serum is an indicator of the adequacy or inadequacy of nitrogen in the diet and thereby the nutritional status of the animal (Hammond, 1983). Inside the body of animals, cholesterol is synthesized from fatty acids, and its concentration in the serum reflects body fat metabolism (Xuan *et al.,* 2018). It is an essential precursor of steroid hormones, including sex steroids.

The activity of enzymes GOT and GPT also rose gradually and significantly from 10 months till 14-15 months of age, and after that, it remained statistically similar till 18 months of age. The levels of both these enzymes were higher in control than the treatment group from 15-16 months of age onwards with significant ($p < 0.01$) differences in GPT activity, and it also varied significantly around 12-13 months of age (Table 2).

Macro-Micro Minerals Profiles

The mean plasma levels of both calcium and phosphorus increased gradually and significantly with advancing age

Table 1: Influence of age and nutritional supplementation on plasma protein and cholesterol profile in growing crossbred heifers

	Total protein (g/dl)		*Total cholesterol (mg/dl)*	
Age (month)	*Control (n = 12)*	*Treatment (n = 12)*	*Control (n = 12)*	*Treatment (n = 12)*
10	$6.47^{a} \pm 0.21$	$6.68^{a} \pm 0.23$	$53.31^{a} \pm 3.20$	$51.08^{a} \pm 2.47$
11	$6.80^{abc} \pm 0.16$	$6.77^{ab} \pm 0.22$	$60.68^{abc}2.00$	$57.60^{ab} \pm 2.95$
12	$6.88^{abcd} \pm 0.17$	$6.87^{abc} \pm 0.16$	$61.84^{abc} \pm 2.36$	$62.04^{b} \pm 2.45$
13	$6.91^{abcd} \pm 0.09$	$7.11^{abcde} \pm 0.13$	$59.06^{ab} \pm 2.88$	$66.07^{bc} \pm 3.55$
14	$7.02^{bcd} \pm 0.08$	$7.21^{bcde} \pm 0.12$	$64.60^{bc} \pm 2.11$	$74.30^{cde} \pm 3.59$
15	$6.73^{ab} \pm 0.14$	$6.94^{abcd} \pm 0.12$	$68.47^{cd} \pm 2.18$	$72.61^{cd} \pm 2.39$
16	$7.05^{bcd} \pm 0.18$	$7.28^{cde} \pm 0.10$	$74.55^{de} \pm 3.73$	$82.17^{de} \pm 3.18$
17	$7.29^{d} \pm 0.09$	$7.39^{de} \pm 0.09$	$82.37^{e} \pm 3.40$	$80.38^{de} \pm 3.69$
18	$7.23^{cd} \pm 0.09$	$*7.55^{e} \pm 0.10$	$78.08^{e} \pm 2.91$	$83.51^{e} \pm 3.62$

*$p < 0.05$ between subgroups; Means bearing uncommon superscripts within the column differ significantly ($p < 0.05$).

Table 2: Influence of age and nutritional supplementation on the activity of plasma GOT and GPT in growing crossbred heifers

	GOT (IU/L)		*GPT (IU/L)*	
Age (month)	*Control (n = 12)*	*Treatment (n = 12)*	*Control (n = 12)*	*Treatment (n = 12)*
10	$31.94^{a} \pm 3.85$	$26.09^{a} \pm 4.03$	$3.30^{a} \pm 0.40$	$2.24^{a} \pm 0.37$
11	$34.38^{a} \pm 4.22$	$32.70^{a} \pm 3.50$	$3.66^{ab} \pm 0.35$	$2.76^{ab} \pm 0.42$
12	$39.97^{ab} \pm 4.85$	$36.88^{a} \pm 3.65$	$4.51^{abc} \pm 0.54$	$*2.75^{ab} \pm 0.36$
13	$48.84^{bc} \pm 4.89$	$49.46^{b} \pm 4.35$	$5.07^{bc} \pm 0.63$	$*3.07^{ab} \pm 0.46$
14	$55.81^{c} \pm 6.26$	$58.03^{bc} \pm 4.34$	$5.78^{cd} \pm 0.82$	$4.09^{bc} \pm 0.41$
15	$70.11^{d} \pm 3.92$	$64.03^{cd} \pm 3.97$	$7.43^{de} \pm 0.80$	$**4.49^{cd} \pm 0.38$
16	$72.23^{d} \pm 3.64$	$67.78^{cd} \pm 4.71$	$8.10^{e} \pm 0.65$	$**4.99^{cd} \pm 0.56$
17	$71.58^{d} \pm 4.65$	$68.43^{cd} \pm 4.94$	$8.50^{e} \pm 0.62$	$**4.91^{cd} \pm 0.48$
18	$82.20^{d} \pm 4.68$	$74.47^{d} \pm 4.42$	$8.64^{e} \pm 0.53$	$**5.58^{d} \pm 0.52$

*$p < 0.05$, **$p < 0.01$ between subgroups; Means bearing uncommon superscripts within the column differ significantly ($p < 0.05$).

from 10 months till 16–17 months of age in both control and treatment groups, with little higher values in treatment than the control group probably due to additional mineral mixture supplemented. However, such significant variation was noted in the plasma magnesium profile only in the treatment group and for manganese in the control group (Tables 3-4). Plasma manganese concentration was found to be significantly ($p < 0.05$) higher at most intervals in treatment than the control group, while magnesium levels were significantly higher in treatment than the control group only after 16th months of age.

Further, the plasma levels of zinc, iron, copper, and cobalt also showed a rising trend with significant differences between 10th and 12–14th months of age, and thereafter the levels were statistically same in all the groups with slightly higher values in treatment than the control group (Tables 5-6). These macro-micro minerals have been most commonly associated with reproductive performance in dairy animals. The alteration may affect ovarian function through its blocking action on the pituitary gland. This results in prolongation of first estrus and ovulation (Sathish Kumar, 2003). Dairy animals should always be provided adequate amounts of minerals to maximize production and reproduction and minimize health problems (Goff, 1999). Inactive ovaries delayed sexual maturity, and low conception rates have been reported in animals with mineral deficiency conditions. In a field study, when heifers received only 70–80% of their mineral requirements, and serum levels

Table 3: Influence of age and nutritional supplementation on plasma calcium and phosphorus levels in growing crossbred heifers

	Calcium (mg/dl)		*Phosphorus (mg/dl)*	
Age (month)	*Control (n=12)*	*Treatment (n=12)*	*Control (n=12)*	*Treatment (n=12)*
10	$8.02^{a} \pm 0.17$	$8.46^{a} \pm 0.15$	$3.25^{a} \pm 0.12$	$3.42^{a} \pm 0.09$
11	$8.67^{b} \pm 0.13$	$*9.22^{b} \pm 0.17$	$3.70^{b} \pm 0.10$	$3.84^{ab} \pm 0.12$
12	$8.74^{b} \pm 0.21$	$9.41^{bc} \pm 0.27$	$4.00^{bc} \pm 0.19$	$3.91^{bc} \pm 0.19$
13	$9.37^{c} \pm 0.22$	$9.69^{bcd} \pm 0.17$	$3.70^{b} \pm 0.14$	$*4.07^{bc} \pm 0.15$
14	$9.97^{d} \pm 0.22$	$10.04^{cde} \pm 0.18$	$4.06^{bcd} \pm 0.20$	$4.38^{cd} \pm 0.21$
15	$10.05^{d} \pm 0.13$	$10.27^{de} \pm 0.14$	$3.99^{bc} \pm 0.14$	$4.29^{bc} \pm 0.15$
16	$10.12^{d} \pm 0.17$	$10.54^{e} \pm 0.26$	$4.35^{cd} \pm 0.14$	$4.36^{cd} \pm 0.11$
17	$10.83^{e} \pm 0.21$	$11.30^{f} \pm 0.30$	$4.48^{d} \pm 0.13$	$4.78^{de} \pm 0.18$
18	$10.94^{e} \pm 0.16$	$*11.55^{f} \pm 0.28$	$4.47^{d} \pm 0.12$	$**4.89^{e} \pm 0.14$

*$p < 0.05$, **$P < 0.01$ between subgroups; Means bearing uncommon superscripts within the column differ significantly ($p < 0.05$).

Table 4: Influence of age and nutritional supplementation on plasma magnesium and manganese levels in growing crossbred heifers

	Magnesium (mg/dL)		*Manganese (ppm)*	
Age (month)	*Control (n = 12)*	*Treatment (n = 12)*	*Control (n = 12)*	*Treatment (n = 12)*
10	2.12 ± 0.12	2.09 ± 0.07^{a}	0.15 ± 0.01^{ab}	*0.19 ± 0.01
11	2.05 ± 0.10	2.16 ± 0.07^{a}	0.14 ± 0.01^{a}	*0.18 ± 0.01
12	2.08 ± 0.10	2.18 ± 0.07^{ab}	0.18 ± 0.01^{c}	0.20 ± 0.01
13	2.05 ± 0.08	2.18 ± 0.07^{ab}	0.17 ± 0.00^{c}	*0.20 ± 0.01
14	2.09 ± 0.06	2.24 ± 0.06^{ab}	0.17 ± 0.01^{c}	0.20 ± 0.01
15	2.14 ± 0.10	2.27 ± 0.07^{ab}	0.16 ± 0.01^{bc}	*0.20 ± 0.01
16	2.04 ± 0.07	$*2.13 \pm 0.08^{b}$	0.18 ± 0.00^{c}	*0.21 ± 0.01
17	2.11 ± 0.08	$*2.40 \pm 0.11^{b}$	0.17 ± 0.01^{c}	*0.20 ± 0.01
18	2.16 ± 0.08	2.29 ± 0.06^{ab}	0.18 ± 0.00^{c}	*0.21 ± 0.01

*$p < 0.05$ between subgroups; Means bearing uncommon superscripts within the column differ significantly ($p < 0.05$).

Table 5: Influence of age and nutritional supplementation on plasma zinc and iron levels in growing crossbred heifers

	Zinc (ppm)		*Iron (ppm)*	
Age (month)	*Control (n = 12)*	*Treatment (n = 12)*	*Control (n = 12)*	*Treatment (n = 12)*
10	0.60 ± 0.04^{a}	$*0.72 \pm 0.04^{a}$	2.15 ± 0.10^{ab}	2.29 ± 0.08^{a}
11	0.62 ± 0.05^{ab}	0.73 ± 0.04^{ab}	2.10 ± 0.11^{a}	2.27 ± 0.09^{a}
12	0.71 ± 0.03^{bc}	$*0.83 \pm 0.03^{bcd}$	2.23 ± 0.10^{ab}	2.49 ± 0.07^{ab}
13	0.78 ± 0.03^{c}	0.79 ± 0.03^{abc}	2.22 ± 0.07^{ab}	$*2.60 \pm 0.10^{bc}$
14	0.72 ± 0.04^{bc}	$*0.85 \pm 0.03^{cd}$	2.20 ± 0.10^{ab}	2.45 ± 0.07^{ab}
15	0.80 ± 0.04^{c}	0.85 ± 0.05^{cd}	2.35 ± 0.10^{ab}	$*2.65 \pm 0.08^{bc}$
16	0.77 ± 0.02^{c}	$*0.87 \pm 0.03^{cd}$	2.44 ± 0.11^{b}	2.62 ± 0.09^{bc}
17	0.80 ± 0.03^{c}	$*0.91 \pm 0.02^{d}$	2.27 ± 0.07^{ab}	$*2.56 \pm 0.12^{bc}$
18	0.79 ± 0.03^{c}	$*0.93 \pm 0.03^{d}$	2.32 ± 0.07^{ab}	**2.77 ± 0.07c

*$p < 0.05$, **$p < 0.01$ between subgroups; Means bearing uncommon superscripts within the column differ significantly ($p < 0.05$).

Table 6: Influence of age and nutritional supplementation on plasma copper and cobalt levels in growing crossbred heifers

	Copper (ppm)		*Cobalt (ppm)*	
Age (month)	*Control (n = 12)*	*Treatment (n = 12)*	*Control (n = 12)*	*Treatment (n = 12)*
10	$0.57^{a} \pm 0.02$	$*0.70^{a} \pm 0.04$	0.39 ± 0.02	$0.39^{a} \pm 0.01$
11	$0.67^{bc} \pm 0.02$	$*0.76^{abc} \pm 0.03$	0.39 ± 0.01	$0.42^{ab} \pm 0.01$
12	$0.70^{bc} \pm 0.02$	$0.77^{abc} \pm 0.04$	0.41 ± 0.02	$0.46^{bcd} \pm 0.02$
13	$0.66^{b} \pm 0.03$	$0.76^{ab} \pm 0.02$	0.43 ± 0.02	$**0.53^{e} \pm 0.02$
14	$0.72^{bcd} \pm 0.04$	$0.78^{abc} \pm 0.02$	0.41 ± 0.02	$0.46^{bcd} \pm 0.02$
15	$0.72^{bcd} \pm 0.04$	$*0.85^{cd} \pm 0.03$	0.41 ± 0.03	$0.44^{abc} \pm 0.03$
16	$0.71^{bc} \pm 0.04$	$*0.86^{cd} \pm 0.02$	0.42 ± 0.02	$0.50^{de} \pm 0.02$
17	$0.76^{cd} \pm 0.03$	$*0.83^{bcd} \pm 0.02$	0.42 ± 0.03	$0.48^{cde} \pm 0.02$
18	$0.80^{c} \pm 0.02$	$*0.89^{d} \pm 0.02$	0.42 ± 0.02	$0.50^{cde} \pm 0.02$

*p < 0.05, **p < 0.01 between subgroups; Means bearing uncommon superscripts within the column differ significantly (p < 0.05).

were low, fertility was impaired (Velladurai *et al.,* 2016). The animals exhibit the delayed onset of puberty and silent or irregular estrus in heifers, failure of estrus from deficient areas. Reduced fertility and reduced or delayed puberty and sexual maturity are the prime signs of deficiency, and this can be overcome with proper mineral supplementation (Sathish Kumar, 2003; Velladurai *et al.,* 2016).

Actual Age at Puberty and Sexual Maturity in Crossbred Heifers

Among 12 heifers, each covered in control and treatment groups, two in each group were excluded for either consistent anestrus with small smooth inactivity ovaries and/or large flat flabby ovaries till 26 months of age. The mean age at puberty for the remaining ten animals in control and experimental groups was 22.23 ± 0.45 and 20.40 ± 0.45 months and sexual maturity (fertile estrus) 24.72 ± 0.89 and 23.17 ± 0.60 months, respectively. These data indicate that a high plane of nutrition from an early age shortens the age at puberty and sexual maturity by nearly two months in HFxK crossbred heifers.

Conclusion

From the study, it is concluded that optimally fed crossbred heifers right from early age attain puberty and sexual maturity with required body weight and plasma biochemical profile of adult animals at around 18 months of age, which is 2-3 months earlier than routine farm fed heifers. Efficient production in domestic animals requires that the essential nutrients in a diet be provided in appropriate amounts and in forms that are most biologically useful to avail of their benefits.

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