Plasma Amino Acid Status of Crossbred Heifers Fed Two Levels of Dietary Protein and its Relationship to Puberty Onset

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ABSTRACT : Twelve prepubertal Karan Fries heifers (15 months, 167.7±13.5 kg) were divided into two equal groups. Group 1 was fed as per NRC requirements and group 2 was fed 20% more protein than group 1 heifers. The experimental feeding was continued until the onset of puberty in both the groups. Blood samples were collected at fortnightly intervals and analyzed for amino acids using HPLC. Group 1 and 2 heifers required 178.6±33.8 and 152.8±33.2 days of experimental feeding to exhibit first estrus resulting in total age at puberty as 639.4±27.3 and 618.6±24.6 days in the two groups respectively. The concentration of total amino acids averaged 4.40 and 4.89 mmol/l and those of non-essential amino acids (NEAA) was 2.32 and 2.49 mmol/l in groups 1 and 2, respectively. The concentration of plasma essential amino acids i.e. histidine, threonine, valine, methionine, isoleucine, leucine and phenylalanine were higher ($p<0.01$) in group 2 than group 1. Plasma concentration of large neutral amino acids (LNAA) was significantly higher in group 2 (1.28 mmol/l) than in group 1 (1.12 mmol/l). Increased levels of leucine, isoleucine and valine are implicated in increased follicular growth and development in prepubertal heifers and resulted in a 26 day earlier attainment of puberty by 26 days in an experimental period of six months in group 2 heifers. Increased concentrations of aspartate and tyrosine in group 2 heifers might be associated with the release of GnRH from the hypothalamus influencing LH release from anterior pituitary in such animals. It is therefore evident that increased availability of certain amino acids in heifers fed high protein diet might have led to early onset of puberty. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 12 : 1714-1718)***

Key Words : Amino acids, Protein, Puberty, Heifers, Tyrosine, Aspartate

INTRODUCTION

Nutrition during the rearing period, through its effects on body weight and backfat levels, can influence the age at which puberty is attained. Severe protein restriction or an amino acid imbalance will significantly delay the age at which a heifer reaches puberty. Therefore, it is important that producers should feed adequate amounts of protein supplements during the developmental period to ensure that puberty is not delayed. A delay of 210 days in the attainment of puberty in Murrah heifers was reported due to feeding of 37% less CP and 16% less TDN (Kaur and Arora, 1989). Lohakare et al. (2001) did not observe any beneficial effect of feeding 25% more protein to heifers on puberty onset. However, there are number of reports indicating the beneficial effects of feeding more protein on early onset of puberty (Hall et al., 1995; Kaur and Arora, 1995). The mechanism by which protein nutrition influences the puberty onset is not clear. Different dietary protein regimens influence the intermediary metabolism leading to variations in glucose, non-esterified fatty acids and urea concentration (Patil, 1993). The availability of different amino acids also differs on different protein intakes and can also influence the animal performance. Sniffen and Jacobson (1975) reported higher absorption rates of amino acids in steers fed 18.8% CP as compared to those fed 13.9% CP in the diet. Literature reveals that blood amino acids influence the reproductive performance of animals through changes in hypothalamic activity and secretion of GnRH (Schillo et al., 1992) or in the pituitary response to GnRH (Deligeorgis et al., 1996). Rats supplemented with tyrosine reached puberty at an earlier age than their counterparts which were not supplemented with this amino acid (Hammerl and Russe, 1987). Ewes fed normal protein diets were reported to be deficient in plasma aspartic acid level and supplementation of this amino acid led to increased gonadotropin secretion and thereby early onset of puberty (Downing et al., 1996). The present study was therefore conducted to observe the effect of feeding high protein diet to prepubertal crossbred heifers on their amino acid status and puberty onset.

MATERIALS AND METHODS

Twelve prepubertal Karan Fries (Sahiwal×Holstein Friesian) heifers about 14-15 months of age were distributed into two equal groups on the basis of their body weight such that body weight at the start of the experiment averaged 169.33±18.34 and 166.17±21.44 kg in groups 1 and 2, respectively. The heifers of group 1 were fed a growing ration as per NRC (1989) recommendation. The heifers in group 2 were fed 20% more CP than group 1. Based on estimated CP content and reported TDN values (Ranjhan, 1980) of green fodder (Table 1) and concentrate

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Table 1. Percent chemical composition (% DM) of green fodders offered

Experimental period (weeks)	Fodder	DM (%)	CP (%)	TDN (%)
0-3	Oats	14.31	13.73	60.0
4-6	Oats	20.27	12.61	60.0
7-10	Oats	24.47	10.87	60.0
11-20	Maize	17.76	14.00	60.0
21-completion	Jowar	25.04	12.50	60.0

ingredients, two diets were formulated to create variability in CP level. Concentrate mixtures 1 and 2 were fed to groups 1 and 2 respectively. The ingredients and chemical composition of concentrate mixture fed to different groups is presented in Table 2. The heifers were housed and fed individually in well-ventilated sheds. Weighed amounts of all the feeds were given to heifers and residues were recorded daily. Fortnightly adjustment in feed supply was done with the change in the body weight of heifers. Daily DM, CP and TDN intake of the animals was calculated. The experiment was continued till the onset of puberty. Blood samples were collected from all the heifers at fortnightly intervals throughout the experimental period. Plasma was separated by centrifugation under refrigeration and was stored at -20°C for further analysis. Amino acid composition of feeds and plasma samples was determined as per the modified PICO-TAG method of Copyright Millipore Corporation (1987). Statistical analysis of the data was carried out according to Snedecor and Cochran (1980) to interpret the effect of dietary protein on amino acid status of heifers and association of various amino acids with the onset of puberty.

RESULTS AND DISCUSSION

Amino acid composition of feeds and fodders offered to experimental animals is presented in Table 3. Concentration of nearly all amino acids was greater in concentrate mixture 2 as compared to concentrate mixture 1. Amino acid concentrations of green maize, green oats and green jowar were almost similar. The total amino acid intake averaged 492.9 g/d in group 1 and 471.6 g/d in group 2 heifers. Essential amino acids (EAA) and non-essential amino acids (NEAA) intakes were calculated to be 194.1 and 298.7 g/d in group 1 and 251.0 and 220.6 g/d in group 2.

Table 2. Ingredient and chemical composition of experimental feeds

Ingredient composition (%)	Concentrate mixture	
	1	2
Maize grain	20	25
Barley grain	25	-
Wheat bran	20	20
Groundnut cake	20	45
Cotton seed cake	12	7
Mineral mixture	2	2
Common salt	1	1
Chemical composition (% DM)		
CP	17.90	24.06
TDN	70.15	69.40
Ca	0.596	0.581
P	0.469	0.472

Mean daily DM, CP and TDN intakes/100kg BW during the experimental period are presented in Table 4. DM and TDN intakes were similar in both the groups. As envisaged, the CP intake remained 21.57 percent higher in group 2 as compared to group 1. Group 2 heifers required 152.8±38.13 days experimental feeding to exhibit first estrus against 178.6±33.78 days in group 1 heifers. The mean age and weight at first estrus was 618.6±24.61 days age and 296.4±5.34 kg BW in group 2 and the corresponding figures in group 1 were 639.4±27.32 days and 288.8±1.74 kg, respectively. Even though the body weight and age at puberty of the two groups did not differ significantly, still there was a decrease of 20.8 days in the attainment of puberty in group 2 heifers due to high protein feeding for about 150 days which bears great practical importance. It is well documented that age at puberty is lower in rapidly growing animals (Kaur and Arora, 1989). Feeding high amounts of protein or excess degradable protein adversely affected animal reproduction (Jordan et al., 1983) due to more ammonia or urea formation in the rumen. Ferguson et al. (1988) suggested that dietary protein intake producing more than 20 mg urea/100 ml serum had an adverse effect on reproduction. However, in the present study, there was no adverse effect of feeding high protein to heifers.

The plasma concentrations (mmol/l) of aspartic acid, histidine, threonine, tyrosine, valine, methionine, isoleucine and phenylalanine were significantly higher ($p<0.01$) in group 2 than group 1 heifers (Table 5). Plasma concentrations (mmol/l) of glutamic acid, serine, glycine, arginine, alanine, proline, cystine and lysine were not affected by dietary treatment. In the present study, the plasma concentrations of total amino acids were 4.40 and 4.89 mmol/l in groups 1 and 2, respectively (Table 6). Though the concentrations were not significantly different ($p>0.05$), still an increase of 0.49 mmol/l in group 2 is considerable because the variations in dietary supply have relatively little effect on plasma amino acid concentration in the ruminants due to microbial protein degradation and resynthesis in the rumen.

The higher essential amino acid intake (g/d) in group 2 (251.0) over group 1 (194.1) might have resulted in increased plasma essential amino acids (EAA) in group 2 (Table 6). Increase in plasma essential amino acids from 0.46 to 0.78 mmol/l was reported due to increase in dietary protein from 5 to 10 % of DM in sheep (Riis, 1983). Similar increase in EAA concentration of lactating cows from 0.84 to 1.02 mmol/l has been recorded by increasing dietary

Table 3. Amino acid composition (g/kg) of experimental feeds

Amino acid	Concentrate mixture 1	Concentrate mixture 2	Green maize	Green oats	Green jowar
Aspartic acid	4.94	13.70	5.42	4.74	4.56
Glutamic acid	17.16	32.81	9.59	8.65	8.76
Serine	7.02	11.41	4.28	4.36	4.51
Glycine	10.19	15.09	4.48	4.13	3.83
Histidine	2.24	1.51	0.62	0.43	0.37
Arginine	4.39	7.80	2.67	1.96	1.99
Threonine	7.84	12.61	5.64	4.24	4.29
Alanine	8.38	3.21	3.78	2.70	2.62
Proline	2.79	1.15	0.99	0.35	0.29
Tyrosine	2.46	3.25	0.86	0.46	0.43
Valine	6.56	7.64	3.12	2.54	2.57
Methionine	2.07	2.67	0.67	0.49	0.48
Cystine	0.76	0.98	0.73	0.64	0.56
Isoleucine	4.32	11.88	2.58	2.32	2.26
Leucine	7.56	7.13	5.29	4.90	4.83
Phenylalanine	5.33	6.21	1.28	1.26	1.11
Lysine	3.68	4.63	2.24	1.95	2.05
Total	97.69	143.68	54.24	46.12	45.51
Non-essential	51.24	78.35	29.27	25.57	25.13
Essential	46.45	65.33	24.97	20.55	20.38

protein from 15 to 18% of total diet DM (Riis, 1983).

The feed supply of non-essential amino acids was 35 to 42 percent higher in group 2 than group 1 (298.7 vs 220.6 g/d), whereas, the plasma amino acid concentration was similar in both the groups (2.32 vs 2.49 mmol/l, Table 6). This indicated that concentration of non-essential amino acids had no consistent relation to feed protein level. Such variability in plasma non-essential amino acid concentration in response to supply of different nitrogen sources was also reported by Riis (1983).

The plasma concentration (mmol/l) of branched chain amino acids like leucine, isoleucine and valine during prepubertal period was significantly higher ($p<0.01$) in group 2 than group 1 (Table 4). Increased blood levels of these amino acids might have supplied more energy substrates to the follicles resulting in follicular growth and development (Downing et al., 1995). Increased blood levels of leucine, isoleucine and valine either due to infusion or feeding lupin grains to ewes have resulted in increased ovulation rates (Smith et al., 1992; Downing, 1994). The infusion of branched chain amino acids provides more gluconeogenic precursors that are utilized for the synthesis of glucose which might be responsible for the increased follicular growth and development.

Table 4. Nutrients intake and onset of puberty in crossbred heifers

Parameter	Group 1	Group 2
DM intake* (kg/100 kg b.wt.)	2.71±0.01	2.73±0.01
CP intake (g/100 kg b. wt.)	405.12^{a}±1.87	471.82^{b}±4.79
TDN intake (kg/100 kg b.wt.)	1.74±0.01	1.74± 0.01
Age at start of experiment (days)	460.8±9.2	465.4±14.5
No. of days in experiment	178.6±33.8	152.8±38.1
Age at onset of puberty (days)	639.4±27.3	618.6±24.6

* Includes intake both from concentrate mixture and fodder.

Values bearing ab superscripts in a row differ significantly ($p<0.05$).

The plasma tyrosine concentration was significantly higher ($p<0.01$) in group 2 than group 1. Higher plasma tyrosine concentration was reported to increase the hypothalamic concentration of tyrosine which is likely to be associated with the hypothalamic release of GnRH (Remirez et al., 1985; AcWorth et al., 1988; Schillo, 1992) in rats. Supplementation with tyrosine resulted in decreased age at puberty in rats (Hammerl and Russe, 1987).

Hall et al. (1992) observed a small increase in LH pulse frequency due to tyrosine infusion whereas, the magnitude of this increase was more in lambs receiving increased feed, indicating that tyrosine alone was not responsible for LHRH mediated LH release. In the present study, tyrosine and other amino acids might be associated with the increased LH secretion leading to early onset of puberty instead of only tyrosine supplementation as was observed in case of rats (Hammerl and Russe, 1987). The increases in LH concentration (1.74 vs 1.16 ng/ml) and LH pulses/6 h (3.29 vs 2.42) were observed in group 2 heifers as compared to group 1 heifers (Swain, 1996).

The dicarboxylic amino acids of aspartate and glutamate often referred to as neuroexcitatory amino acids, act as neurotransmitters in the central nervous system. The level of aspartate in the present experiment was significantly higher ($p<0.01$) in group 2 than group 1, whereas, glutamate concentration was similar in both the groups. The injection of N-methyl aspartate (NMA), a potent agonist of aspartate and glutamate stimulated LH release (Downing et al., 1996) whereas injection of its antagonist resulted in decreased LH pulse frequency and pulse amplitude (Shahab et al., 1995). The increased aspartate concentration in group 2 heifers (Table 5) might have influenced GnRH releasing

Table 5. Effect of dietary protein on plasma amino acid concentration (mmol/l) of heifers

Parameters	Group 1	Group 2
Aspartic acid	0.40^{a}±0.01	0.45^{b}±0.01
Glutamic acid	0.59±0.02	0.61±0.02
Serine	0.36±0.01	0.38±0.01
Glycine	0.25±0.07	0.26±0.01
Histidine	0.10^{a}±0.00	0.17^{b}±0.01
Arginine	0.24±0.01	0.26±0.01
Threonine	0.31^{a}±0.01	0.36^{b}±0.01
Alanine	0.25±0.02	0.26±0.02
Proline	0.12±0.01	0.14±0.01
Tyrosine	0.18^{a}±0.00	0.21^{b}±0.00
Valine	0.29^{a}±0.01	0.32^{b}±0.01
Methionine	0.15^{a}±0.00	0.19^{b}±0.00
Cystine	0.16±0.00	0.16±0.00
Isoleucine	0.15^{a}±0.00	0.18^{b}±0.00
Leucine	0.33^{a}±0.01	0.37^{b}±0.01
Phenylalanine	0.17^{a}±0.01	0.22^{b}±0.02
Lysine	0.32±0.01	0.34±0.00

Values bearing ab superscripts in a row differ significantly ($p<0.01$).

Table 6. Essential, non-essential and neuroexcitatory amino acids (mmol/l) in prepubertal heifers

Parameters	Group 1	Group 2
Plasma total amino acids	4.40±0.14	4.89±0.21
Essential amino acids	2.08^{a}±0.06	2.41^{b}±0.10
Non-essential amino acids	2.32±0.08	2.49±0.10
Large neutral amino acids	1.12^{a}±0.03	1.28^{b}±0.06
TYR:LNAA ratio	0.16±0.00	0.16±0.00
Neuroexcitatory amino acids	0.99±0.03	1.07±0.04

Values bearing ab superscripts in a row differ significantly ($p<0.05$).

neurons in hypothalamus, which in turn might be involved in the maintenance of the pulsatile secretory pattern of LH leading to early sexual maturity. Hence, it can be inferred that decrease in the age of onset of puberty in heifers fed high protein diet might be due to more availability of certain amino acids which are implicated either in increased energy availability or associated with the pulsatile release of LH required for puberty onset.

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