PLASMA CONCENTRATION OF L. THYROXINE (T_4), L. TRIIODOTHYRONINE (T_3) AND CERTAIN OTHER BLOOD BIOCHEMICAL CONSTITUENTS IN GROWING CROSSBRED (*Bos taurus* × *Bos indicus*) CALVES

D. C. Shukla[1], V. P. Varshney, P. S. P. Gupta and B. B. Mahapatro

Division of Physiology and Climatology Indian Veterinary Research Institute, Izatnagar (U.P.) 243122, India

Summary

The relationship of thyroid status and certain blood biochemical constituents with body weight gains (ADG) and age (13 to 96 weeks) was studied in Holstein Friesian × Hariana (1/2F × 1/2H) crossbred male calves by assessing their plasma triiodo thyronine (T_3), thyroxine (T_4), sodium, potassium, total proteins and cholesterol level at two energy levels. Body weight gains (ADG) were higher during the 50 to 72 weeks of age and declined thereafter. The plasma T_3 conc. was significantly ($p < 0.01$) higher during this period compared to all other periods. There was no significant variation due to energy level. Overall mean plasma T_3 conc. was $1.19 + 0.12$ ng/ml. Plasma T_4 conc. did not show any significant variation either between the different age periods or between the two energy levels. The mean plasma T_4 conc. was 37.34 ± 1.32 ng/ml.

The plasma sodium and potassium concentration did not vary significantly due to energy levels. But amongst the different age periods, sodium concentration was highest (147.70 ± 2.29 mEq/L) during 49-60 weeks of age and lowest (134.70 ± 1.78 mEq/L) during 13-24 weeks, where as for potassium concentration changes were nonsignificant. There was very little variation amongst other periods. Plasma protein level was higher at 100% energy level than at 75%. Amongst the different age period, it was significantly lower (6.44 ± 0.36) during 13 to 24 weeks of age than at 37 to 48 weeks of age(7.14 ± 0.11). Plasma cholesterol values were higher for 75% energy level than that of 100%. Between the perioids it was highest during 61 to 72 weeks of age and the differences amongst the age periods were highly significant.

(**Key Words** : Crossbred, Blood Constituents, Growth, Thyroid)

Introduction

Thyroid hormones have been shown to control many physiological function including cellular metabolsim (McDonald, 1980). Efforts have been made to study the effect of season, age, breed, pregnancy and lactation (Henneman, et al., 1955; Khub and Goel, 1984; Refsal, 1984). Serum L. thyroxine (T_4) level has been reported to decrease with age in both the sexes, but L. triiodothyronine (T_3) decreases with advancing ages in male rats only (Tong, 1985).

Serum protein values have been reported for calves (Shannan and Lascelles, 1966; Dalton, 1967) and cows (Dimopoullos, 1961; Tashjian et al., 1968). Also the serum protein concentration as a function of age has been reported for dairy cattle (Larson and Touchberry, 1959; Mylrea and Healy, 1968). Likewise plasma cholesterol concentration has been reported for dairy cattle in various indigenous and exotic breeds. Blood electrolyte balance has been studied in dairy cattle by many workers (Mc-Sherry and Grinyer, 1954; Fisher, 1960; Tumbleson, 1973). However, very little information is available on changes in plasma thyroxine, triiodothyronine level and the blood biochemical constituents level due to age in crossbred calves (*Bos taurus* × *Bos indicus*) and no information is available on relationship of these levels with growth rate and dietary energy level. Therefore, an investigation was made on plasma levels of these constituents in crossbred (Holstein Friesian × Hariana) calves from 13 to 96 weeks of age as related to their growth rate and the dietary energy level.

[1]Address reprint requests to Dr. D. C. Shukla, Senior Scientist, Division of Physiology & Climatology, Indian Veterinary Research Institute, Izatnagar (U.P.) 243122, India.

Received March 19, 1993
Accepted September 18, 1993

Materials and Methods

Experimental animals and dietary regimen

Ten crossbred (Holstein Friesian × Hariana) male calves (12 weeks old) received from Livestock Production Research (Cattle and Buffalo) Division of this Institute were used in this investigation. The calves were put either on standard ration as per Kearl (1982) or on 25% less energy ration. Digestible crude protein was provided to meet out full protein requirement to both the groups. Thus one group was provided 100 percent (E.L.I) and the other group 75 percent of its energy requirement (E.L.II). Concentrate mixture and roughage was provided to meet out their nutrient requirements for body weight gain of 500 g/day. The concentrate to roughage ratio was 50:50.

TABLE 1. COMPOSITION OF RATION

	Concentrate		Roughage
	Energy level I	Energy level II	
Physical			
– Groundnut cake	20	45	
– Crushed maize	40	25	Wheat straw
– Wheat bran	37	27	
– Min. mixture	2	2	
– Salt	1	1	
Chemical	g/100 g	D.M. basis	
– Moisture	8.0	5.0	1-2
– Crude protein	17.85	22.58	2.50
– Ether extract	2.57	3.10	0.08
– Crude fibre	7.95	8.58	39.00
– Energy contents (MJ/kg DM)	18.69	18.56	17.70

Concentrate mixture was supplemented with neuvimin Forte (M/s Sarabhai, India) @ 250 g/100 kg for mineral and vitamins and Vitablend (M/s Glaxo, India) for Vitamin A supplement.

Hormone assay

Heparinised blood samples were collected from Jugular vein every fortnightly at 9.0 A.M. until 96 weeks of age from all the calves and plasma was separated out and preserved at −20°C ± 5°C untill analysis. L. triiodothyronine (T_3) and L. thyroxine (T_4) were estimated by radioimmunoassay methods (Bhandarkar and Pillai, 1982) using RIA kit (Bhabha Atomic Research Centre, Bombay, India). Intra and inter assay coefficients of variations were recorded as 8.6 and 11.8% for T_3 and 9.2 and 12.2% for T_4 respectively.

Chemical analysis

Total proteins were estimated by the method of Reinhald (1953) and cholesterol was estimated by the method of Watson (1960). Sodium and potassium concentrations were estimated as described by Oser (1965) using 'Biochem' digital flame photometer.

Statistical design and analysis

The data were classified into six age periods representing 25-36, 37-48, 49-60, 61-72, 73-84 and 85 to 96 weeks of age for T_3 & T_4 whereas for other parameters with an additional age period of 13 to 24 weeks, analysis was made between seven periods, for the differences due to age and energy level. The experiment was conducted following the randomised block design. The data were analysed by analysis of variance (Snedecor and Cochran, 1967) and the differences between the group means were tested by Duncan's multiple range test (Steel and Torrie, 1960).

TABLE 2. CONCENTRATION OF TRIIODOTHYRONINE (T_3) AND THYROXINE (T_4) IN BLOOD PLASMA

Age periods (weeks)	Triiodothyronine (T_3) (ng/ml)	Thyroxine (T_4) (ng/ml)
25 to 36	0.56 ± 0.07^a	35.33 ± 2.68
37 to 48	0.77 ± 0.09^a	37.82 ± 2.87
49 to 60	2.54 ± 0.04^b	35.71 ± 2.41
61 to 72	1.54 ± 0.31^c	39.69 ± 3.46
73 to 84	0.82 ± 0.07^a	42.13 ± 4.02
85 to 96	0.88 ± 0.05^a	$35.59 + 2.46$
100% energy (level I)	1.25 ± 0.16^c	35.95 ± 1.50
75% energy (level II)	1.14 ± 0.18^c	37.07 ± 2.01

Means with common superscripts are not significantly different.

Results and Discussion

The body weights were recorded fortnightly and cumulative body gains calculated as average daily gains (ADG) g/day for the entire period of the study (24 to 96 weeks of age). ADG for the period I, II, III, IV, V and VI were 422 ± 10.32, 510 ± 7.2, $505 + 11.64$, 501 ± 7.21, and 439 ± 13.90 at 100% and 398 ± 11.91, 479 ± 12.90, 497 ± 16.50, 513 ± 12.41, 497 ± 11.40 and 389 ± 12.30 g at 75% energy levels, respectively. Thus, it is evident from these values that the maximum body gains were obtained during the 61 to 72 weeks of age at both the energy levels.

The values of plasma T_3 and T_4 conc. (ng/ml, $\bar{X}$ & SE) for the different age periods and energy levels are given in table 2. It is evident from these values that the thyroid activity as expressed by plasma T_3 and T_4 levels changed with the age of calves. Plasma triiodothyronine (T_3) values varied from 0.72 ± 0.08 to 2.61 ± 0.45 ng/ml in E.L. I and 0.46 ± 0.09 to 2.47 ± 0.63 ng/ml in E. L. II. The overall means for the two energy levels were recorded as 1.25 ± 0.16 and 1.14 ± 0.18 ng/ml at E.L. I and II respectively. The plasma T_3 conc. between these two energy levels did not vary significantly. The mean plasma triiodothyronine conc. for the different age periods ranged from 0.56 ± 0.07 to 2.54 ± 0.04 ng/ml. This indicated significant ($p < 0.01$) change in T_3 conc. during different age periods. The lowest conc. was recorded at the age period of 24 to 35 weeks whereas the maximum conc. was recorded between 49 to 60 weeks. The plasma T_3 conc. has shown significant ($p < 0.01$) rise from 25 to 60 weeks and then declined gradually but significantly ($p < 0.01$) through the remaining periods. This rising trend in the plasma T_3 values upto the age of 60 weeks and decline thereafter as evident at both the energy levels in this experiment, indicates that the variations in energy levels used in this study did not effect the trend of thyroid activity except that at E.L. II the T_3 levels have shown a slight rise at the period VI. The highest plasma T_3 conc. was obtained at the age between 49 to 60 weeks at both the energy levels. When this was compared with growth rate (ADG), the two peaks have coincided. The ADG also was highest during the period III and IV and declined therafter. Thus the plasma T_3 conc. seems to be significantly related with the growth rate and age of the animal.

Plasma thyroxine (T_4) conc. varied from 32.34 ± 1.85 (period I at E.L. I) to 43.15 ± 5.44 ng/ml (period V at E.L. II). Plasma T_4 conc. has also varied between the periods at the two energy levels but there was no specific trend. The T_4 conc. shown rise and fall from period to period. There was no significant variations in T_4 conc. amongst the different age periods. However, the variations between the animals were significantly high and that might have been the reason for non significant effect of age on plasma T_4 concentration. Similar observations have been reported for exotic breeds (Curtis and Abrams, 1979; Refsal et al., 1984), as the later workers have recorded an inverse relationship of T_4 and T_3 with age. Oaka-Souda et al. (1977) have also, showed the characteristics feature of plasma T_3 and T_4 conc. with the developmental changes in rats. They reported hormonal peaks at critical periods of rats development and decline thereafter. Tong (1985) also reported decrease in serum T_4 conc. with age in both the sexes while in T_3 conc. age associated diminution in male rats only. However, Irvin and Trenkle (1971) could not record any influence of age or breed on plasma protein bound iodines in the cattle, but Long et al. (1951) have observed marked decline in blood PBI levels with age upto 3-4 years and thereafter a gradual decline.

It is well known that triiodothyronine is physiologically more active than thyroxine and provides better indication of metabolic status of the animal, which has also been reflected in this study. Similar hypothesis has been suggested earliar (Refsal et al., 1984) also for lactating dairy cows.

The mean concentrations ($\bar{X}$ and S.E.) of total proteins, cholesterol, sodium and potassium for the different periods and energy levels, are given in table 3.

The plasma total protein concentration was observed as 6.88 ± 0.24 and 6.50 ± 0.33 g/dl for 100% and 75% energy levels respectively. Thus the calves maintained on 100% energy level had significantly ($p < 0.05$) higher plasma total proteins than the calves on 75% energy level. Amongst the different age periods the protein concentration was lowest (6.44 ± 0.36 g/dl) during the 13 to 24 weeks of age and highest (7.15 ± 0.11 g/dl) during 37 to 48 weeks of age. The variations between the age periods were significant ($p < 0.05$). Dimopoullos (1961) and Tumbleson et al. (1973) reported a linear increase in serum protein contents with age in comparatively older (1 to 10.5 yr) Gurnsey and Holstein cows. The values reported by them were higher than the values observed here for the crossbred calves. However, present values agreed with those of Tashjian et al. (1968). Though the trend of increase in plasma proteins with increasing age was present in this experiment the rise was not as marked as observed by Larson and Touchberry (1959), Riegle and Meller (1966) and Tumbleson et al. (1973).

Mean cholesterol concentrations were 130 ± 10.51 mg/dl and 144.00 ± 8.03 mg/dl in 100% and 75% energy fed animals respectively. Amongst the different age periods, it was lowest (101.20 ± 4.82) during the first period and highest (162.00 ± 7.49) during the V age period. Thus there was a significant ($p < 0.01$) effect of energy level as well as of age periods on the plasma cholesterol concentration. There was a steady increase in its concentration with increasing age upto 72 weeks similar to that as reported earlier (Arave et al., 1975; Rowland, 1980).

Mean plasma sodium concentration was 137.20 ± 2.35 mEq/L and 138.40 ± 1.63 mEq/L at 100% and 75% energy levels respectively and no significant difference was found. Between the different age periods, the calves at the age of 13 to 24 weeks had the lowest (134.70 ± 1.78 mEq/l) sodium concentration and the highest (147.70 ± 2.29 mEq/L) between 49 to 60 weeks.

TABLE 3. MEAN CONCENTRATION ($\bar{X}$ AND S.E.) OF BLOOD CONSTITUENTS FOR DIFFERENT AGE PERIODS

Age periods	Sodium (mEq/L)	Potassium (mEq/L)	Plasma total protein (gm/dl)	Total cholesterol (mg/dl)
13 to 24 weeks	134.70 ± 1.78^{b}	4.85 ± 0.10^{a}	6.44 ± 0.36^{c}	101.20 ± 4.82^{c}
25 to 36 weeks	138.90 ± 1.67^{a}	4.97 ± 0.11^{a}	6.90 ± 0.13^{ac}	113.70 ± 4.84^{c}
37 to 48 weeks	141.30 ± 1.48^{a}	$4.70 + 0.12^{a}$	7.15 ± 0.11^{a}	132.20 ± 5.27^{b}
49 to 60 weeks	147.70 ± 2.29^{c}	5.02 ± 0.14^{a}	6.97 ± 0.17^{ab}	151.00 ± 6.01^{a}
61 to 72 weeks	135.50 ± 2.89^{b}	4.74 ± 0.18^{a}	6.63 ± 0.17^{bc}	162.00 ± 7.49^{a}
72 to 83 weeks	$138.20 + 1.69^{a}$	4.85 ± 0.16^{a}	6.87 ± 0.14^{ac}	156.40 ± 6.72^{a}
84 to 96 weeks	137.80 ± 1.37^{a}	$4.57 + 0.11^{a}$	$6.74 + 0.21^{ac}$	$137.00 + 6.67^{b}$
Energy level				
100%	$137.20 + 2.35^{A}$	4.85 ± 0.10^{A}	6.88 ± 0.24^{B}	130.00 ± 10.51^{C}
75%	138.40 ± 1.63^{A}	$4.78 + 0.06^{A}$	6.50 ± 0.33^{C}	$144.00 + 8.03^{D}$

Means with different superscripts significantly.

The difference between highest and lowest was significant ($p < 0.01$) Tumbleson et al. (1973) have also reported the sodium concentration to vary from 133.8 to 139.9 mEq/L in Gurnsey and Holstein dairy cattle, but did not record any change in sodium concentration in mature animals. The values recorded here are in agreement with these of Evans and Phillipson (1957), Tumbleson et al. (1973) and Rowland (1980).

The mean plasma potassium concentration was 4.85 ± 0.10 and 4.78 ± 0.06 mEq/L for 100 % and 75% energy, respectively and did not differ significantly. At the different age periods potassium concentration varied from 4.57 ± 0.11 to 5.02 ± 0.14 mEq/L but these differences also were not significant. Fisher (1960) has reported significantly higher potassium concentration in Ayrshire calves than in the crossbred calves reported in this study. Little et al. (1977) have reported decreased potassium concentration with age steadily over the period of 2 to 13 weeks of age in Friesian and Ayshire calves.

The electrolyte balance in this study was not found to be affected by the energy level of the diet. Age-dependent changes were found for sodium but not for potassium concentration during the period of age studied here. The concentration of total protein was affected by the level of energy in the diet and to some extent by age. Cholesterol levels on the other hand were affected by the energy level as well as by the age.

Acknowledgement

The authors are thankful to the Director of the institute and Head of the Divison of Physiology and Climatology for providing necessary facilities for the work.

Literature Cited

Arave, C. W., R. H. Miller and R. C. Lamb 1975. Genetic and environmental effect on serum cholesterol of dairy cattle of various ages. J. Dairy Sci. 56:423-427.

Bhandarkar, S. D. and M. R. A. Pillai. 1982. Radioimmunoassay A laboratory maunual-Bhabha Atomic Research Centre, Bombay.

Curtis, R. J. and J. J. Abrams. 1979. Circadian rhythms in the concentraion of thyroid hormones in the plasma of normal animals. British. Vet Jour. 133:134-144.

Dalton, R. C. 1967. Variation in calf plasma composition with age. British Vet. Jour. 125:48-52.

Dimopoullos, C. T. 1961. Polysaccharides and protein relationships of normal bovine sera. American J. Vet. Res. 22 986-989.

Evans, J. V. and A. T. Phillipson. 1957. Electrolyte concentration in erythrocytes of goat and ox. J. Physiol. 139:87-96.

Fisher. E. W. 1960. The concentration of some of the inorganic constituents in the plasma of healthy Ayrshire cattle. Br. J. Nutr. 14:9-12.

Henneman, H. A., E. P. Reineke and S. A. Griffin. 1955. The thyroid secretion rate of sheep as affected by season, age, breed, pregnancy and lactation. J. Anim. Sci. 14:412-434.

Irvin, R. and A. trenkle. 1971. Influence of age, breed, sex on plasma hormones in cattle. J. Anim. Sci. 32:292-795.

Kearl, L. C. 1982. Nutrient requirement of ruminants in developing countries. Int. Feed. Stff Inst Utah.

Khub S. and V. K. Goel. 1984. Thyroid activity in second generation crosses of Hariana cattle with exotic breeds. Indian J. Physiol., Pharmacol. 28: 333-334.

Larson, B. L. and R. W. Touchberry. 1959. Blood serum protein levels as a function of age. J. Anim. Sci. 18:983-990.

Little, W., R. M. Key, R. Manston, G. J. Rowland and A. J. Stark. 1977. The effect of age, live weight gain and feed intake on the blood composition of young calves. J. Agric. Sci. (Cambridge) 89:291-296.

Long, J. F., L. O. Gilmore, C. M. Curtis and D. C. Rife. 1951. The bovine PBI as related to age, sex and breed. J. Anim. Sci. 10:1027-1028.

McDonald, L. E. 1980. Veterinary Endocrinology and Reproduction. 3rd edn., K. M. Varghes & Co. Bombay, India. 45-57.

Mc-Sherry, B. J. and I. Grinyer. 1954. The pH values, carbondioxide contents and the levels of sodium, potassium, calcium, chloride and inorganic phosphorus in the blood serum of normal cattle. American J. Vet. Res. 15:509-570.

Mylrea, P. J. and P. J. Healy. 1968. Concentration of some components in the blood and serum of apparently healthy dairy cattle. 1. Electolytes and minerals. Australian Vet. Jour. 44:565-569.

Oaka-Souda, S., D. J. Draves and P. S. Timiras. 1977. Developmental patterns of plasma TSH, T_4 and T_3 in rats deprived of light from birth. Mechanism Aging Development, 6:287-291.

Oser, B. L. 1965. Hawk's Physiological Chemistry 14 th edn. London. McGraw-Hill Company.

Refsal, K. R., R. F. Nachreiner and C. R. Anderson. 1984. Relationship of season, load, lactation, age and pregnancy with serum thyroxine and triiodothyronine in Holstein cows. Domest. Anim. Endocr. 1:225-235.

Reinhold, J. G. 1953. Total protein, albumin and globulin. In standard methods of clinical chemistry (ed. M. Reiner) Vol. I. pp. 88-97. New York, Academic Press.

Riegle, G. D. and J. W. Mellor. 1966. Change in blood cellular and protein components during aging. J. Gerantology. 21:435-436.

Rowland, G. J. 1980. A review of variations in the concentrations of metabolites in the blood of beef and dairy cattle associated with physiology, nutrition and disease with particular reference to interpretation of metabolic profile. World Review of Nutrition and Dietics. 35:172-235.

Shannan, A. D. and A. J. Lascelles. 1966. Change in the concentration of lipids and some other constituents in the blood plasma of calves from birth to 6 months of age. Australian Journal of Biological Sciences. 19:831-839.

Snedecor, C. W. and W. C. Cochran. 1967. Statistical methods: 6th edn., Oxford IBM Publishing Co., New Delhi.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc. New York, USA.

Tashjian, R. J., J. W. Snyder and D. M. Das. 1968. Blood studies of 32 clinically normal Ayrshire cattle. Cornell Veterinarian. 58:8-11.

Tong, F. 1985. *Effect of sex and age on serum aldo*esterone and thyroid hormones in the laboratory rats. Hormone Metabolism Research. 17:507-509.

Tumbleson, M. E., W. E. Ulingfield, H. D. Johnson, J. R. Campbell and C. C. Middleton. 1973. Serum electrolytes concentration as a function of age in female dairy cattle. Cornell Veterinarian. 63:58-61.

Watson, D. 1960. A simple method for determination of serum cholesterol. Clinical Chem. Acta 5:637-643.