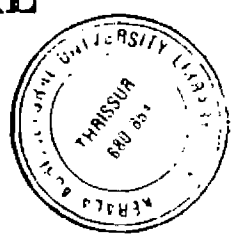
EVALUATION OF CERTAIN METABOLIC AND HAEMATOLOGICAL PARAMETERS IN CROSSBRED CALVES FED WITH RUMEN UNDEGRADABLE SOYABEAN MEAL



By

N. YUVARAJ

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Physiology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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DECLARATION

I hereby declare that this thesis entitled **"EVALUATION OF CERTAIN METABOLIC AND HAEMATOLOGICAL PARAMETERS IN CROSSBRED CALVES FED WITH RUMEN UNDEGRADABLE SOYABEAN MEAL"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

19/10/02

Mannuthy

N. YUVARAJ

CERTIFICATE

Certified that the thesis entitled **"EVALUATION OF CERTAIN METABOLIC AND HAEMATOLOGICAL PARAMETERS IN CROSSBRED CALVES FED WITH RUMEN UNDEGRADABLE SOYABEAN MEAL"** is a record of research work done independently by **Dr. N. Yuvaraj**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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We, the undersigned members of the Advisory Committee of **Dr. N. Yuvaraj,** a candidate for the degree of Master of Veterinary Science in Physiology, agree that the thesis entitled **"EVALUATION OF CERTAIN METABOLIC AND HAEMATOLOGICAL PARAMETERS IN CROSSBRED CALVES FED WITH RUMEN UNDEGRADABLE SOYABEAN MEAL"** may be submitted by Dr. N. Yuvaraj in partial fulfilment of the requirement for the degree.

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N. Yuvaraj

To

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CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

Proteins form one of the most significant constituents of the ruminants' ration and they are generally the most expensive ingredients in their rations. It is, therefore, of paramount importance to ensure that this constituent is utilized with high efficiency. Improvements in animal performance largely depend on decreasing the ruminal degradation of proteins. Availability of nutrients post-ruminally is generally ignored when one considers the efficacy of escaping and absorbing protein and other nutrients in the lower gastrointestinal tract. If the Maillard reaction between sugar aldehyde groups and free amino groups can be enhanced to decrease protein solubility and degradation in the rumen without adversely affecting intestinal protein digestibility, then improved performance in terms of nitrogen retention, weight gain and feed efficiency could be guaranteed (Chalupa, 1975). Thomas *et al.* (1979) pointed out that protection of highly soluble good quality proteins from rumen microbial degradation resulted in more desirable amino acid absorption from the digestive tract.

Post-ruminal utilisation of nutrients eliminates energy losses associated with fermentation and protein losses incurred in the transformation of dietary protein to microbial protein. The efficiency of converting dietary protein into rumen microbial protein in normally fed calves has been estimated to be only slightly above 50 per cent. Moreover, microbial nitrogen is lower in digestibility than many plant protein nitrogen degraded in the rumen since microbial nitrogen contains 10 to 20 per cent non-nutritious nucleic acid

nitrogen. Similarly, protein digestion in the small intestine of ruminants may not be as complete as it is in monogastrics (Chalupa, 1975).

There are various methods of decreasing protein and amino acid degradation in the rumen, which include heat treatment, chemical treatment, encapsulation, use of amino acid analogues and inducing oesophageal groove closure. It is advantageous that these procedures do not interfere with ruminal metabolism or post-ruminal digestion. Out of several protection methods the most promising approach seems to be the modification of proteins by heat and formaldehyde treatments in order to decrease the solubility and hence less susceptibility to ruminal microbial degradation (Chalupa, 1975). Though, it is generally believed that formaldehyde is carcinogenic in nature, feeding of formaldehyde treated diets has never been reported to cause any deleterious effects in animals (McGuffey *et al.*, 1977) and it is favoured because of cost factor. In fact, its use has been legalized in many Western countries. When formaldehyde is mixed with protein, some amount of formaldehyde escapes in gaseous form, leaving behind the remainder to form methylene bridges (-HCH-) with the amino acids. Furthermore, the amount of formaldehyde bound is a measurable parameter that can be used to evaluate the effectiveness of formaldehyde treatment. About 1 to 2 g of bound formaldehyde per 100 g of crude protein appears to be the desired range (Sampath, 1990).

Post-natal growth in ruminants is chiefly influenced by the metabolic hormones viz., somatotropin, thyroid hormones and insulin, which cause direct physiological changes in the metabolism. Although the effects of thyroid

hormones are primarily on cell differentiation in the fetal life, it should be pointed out that maximum growth rate of animals is obtained by the synergetic actions of growth hormone, thyroid hormones and insulin rather than growth hormone alone post-natally. Release of thyroxine (T_4), somatotropin and insulin in high magnitude during the period of growth is critical for guaranteed maximum growth. Higher the availability of dietary proteins to the host, higher the availability of critical amino acids in the circulation, greater will be the amplitude of release of thyroxine, somatotropin and insulin and in turn, greater the resultant growth. Meagre studies have been conducted to evaluate the role of these metabolic hormones and their interrelation with high dietary protein intake in terms of undegradable protein in ruminants.

The objectives of the present study are

(i) to evaluate the physiological changes that could be brought about by feeding concentrate having standard levels of protein and a higher level of rumen undegradable proteins.

(ii) to determine the levels of certain metabolic hormones like triiodothyronine (T_3), thyroxine (T_4) and insulin and other related haemotological and biochemical parameters in crossbred calves fed with concentrate rations containing higher levels of rumen degradable and rumen undegradable (formaldehyde protected) soyabean meal and to find out the correlation, if any, among the above parameters.

Review of Literature

2. REVIEW OF LITERATURE

Proteins form one of the most significant nutrients in the ruminant ration and protein supplements are generally the most expensive ingredients in their rations. Their maximal utilization with high efficiency is of paramount importance and could be achieved by protecting them from degradation in fore-stomachs. Protected proteins escape the fore-stomach (rumen), reach abomasum and thus, the availability of amino acids from dietary protein is increased in abomasum and intestine. The dietary supplemented bypass/ protected protein may improve the performance of the ruminants. The available literature with respect to proteins in the ruminant ration, protection of feed proteins and effects of rumen undegradable proteins on milk yield, reproduction, growth and on certain haematological, biochemical and certain hormonal parameters have been reviewed in this chapter.

2.1 Proteins in the ruminant ration

The dietary crude protein of ruminants can be described in terms of rumen degradable protein (RDP) and undegradable protein (UDP) (Burroughs *et al.*, 1975; Kaufmann, 1977). The ruminants meet their amino acid requirements from the digestion of microbial protein and rumen undegraded dietary protein, which are hydrolysed in the abomasum and small intestine. In order to meet the protein / amino acids requirement of ruminants for high milk yield and growth, Roy *et al.* (1977) proposed new feeding systems by incorporating greater proportion of rumen undegradable dietary protein (UDP)

in the ruminants' ration for high milk yield and growth. Agricultural Research Council (ARC, 1980) and National Research Council (NRC, 1989) proposed that the nitrogen compounds in feedstuffs should be classified in terms of their rumen degradable nitrogen (RDN) and rumen undegradable nitrogen (UDN) contents. Chalupa (1982) reported that nitrogen economy of ruminant animals was dependant upon the proper balance of degradable and undegradable proteins and Kaufmann and Lupping (1982) were of the opinion that the ration of growing calves should contain certain amount of UDP.

In order to overcome the limitations of digestible crude protein (DCP) system, the protein subgroup of the working party of ARC of United Kingdom on nutrient requirement of ruminants had proposed that the dietary crude protein needs of ruminants must be supplied in terms of rumen degradable protein (RDP) which meet the nitrogen requirement of rumen microbes and undegraded dietary protein (UDP) be made available to the host animal whenever the microbial protein synthesis is insufficient to meet the nitrogen requirement (ARC, 1984).

Chalupa (1984) opined that microbial protein was less digestible than undegraded feed protein and according to Waldo and Glenn (1984), the digestibility co-efficient of microbial protein was 0.70 - 0.85 while that of UDP was 0.70 - 0.90. Increased percentage of rumen UDP enhanced the productivity of heat-stressed lactating cows (Higginbotham *et al.*, 1989 and Taylor *et al.*, 1991) and growing calves (Bunting *et al.*, 1992 and White *et al.*, 1992). However, it was not determined whether the apparent benefits were due to the

improved conservation of nitrogen with reduced RDP or due to changes in the amino acid profile of proteins reaching the intestine.

According to De-Jong (1997), higher levels of UDP in the diet resulted in higher contents of utilizable crude protein and they recommended that in order to obtain full revenue benefit, livestock farmer should calculate protein requirement according to the utilizable crude protein content of the compound feed than crude protein content.

2.1.1 Soyabean meal

Soyabean meal is one of the major protein supplements for dairy cows with excellent palatability and higher amino acids availability. Loerch *et al.* (1983) on evaluating digestibility and rumen escape nitrogen content of soyabean meal, blood meal, meat and bone meal and dehydrated alfalfa, found that the digestibility of soyabean meal was significantly ($p<0.012$) greater than others whereas nitrogen escape of soyabean meal was markedly lower ($P<0.05$) than others. Gupta and Gupta (1984) reported the rumen degradable protein (RDP) and rumen undegradable protein (UDP) values of soyabean meal with a rumen outflow rate of 0.05/h as 39 per cent and 5 per cent respectively.

Zerbini and Polan (1985) evaluated protein sources for Holstein calves fed with a basal diet of 11.6 per cent crude protein (CP) and supplemented with the added protein to make a total of 15.5% CP and found that fish meal and soyabean meal generated highest values of gain and were better protein sources for growth than were corn gluteal meal or cotton seed meal.

Johri *et al.* (1988) recorded higher levels of lysine in soyabean meal compared to groundnut cake and mustard cake. The RDP and UDP values of soyabean meal recorded by Negi *et al.* (1989) were 19 per cent and 31 per cent respectively. Soyabean meal possessed a high quality post ruminal essential amino acid index, that was next to only ruminal microbial protein with a relatively lower ruminal undegradable protein of only 28-34% crude protein (NRC, 1989).

Based on several research articles and various *in vivo* methods, *in vitro* procedures and *in situ* techniques including solubility tests conducted, Sampath (1990) concluded that most of the protein sources of vegetable origin were highly degradable in the rumen (61-100 per cent) and the degradability of soyabean meal was 66 per cent with a crude protein, RDP and UDP contents of 50 per cent, 33 per cent and 17 per cent respectively. Sindt (1992) found that finishing calves that received 100 per cent of their supplemental nitrogen from soyabean meal gained faster and more efficiently during the early finishing period than calves that received their supplemental nitrogen from feather meal and urea (50/50) and concluded that an escape nitrogen values of 52 and 92 per cent for soyabean meal and feather meal respectively.

Sibanda *et al.* (1993) reported that soyabean meal apparently complemented the microbial protein and total nutrient supply obtained from the basal diet was better than the other three protein sources tested viz., cotton seed meal, meat and bone meal and blood meal. They also reported a significantly ($P<0.05$) higher body weight gain upon feeding soyabean meal than the other

three protein sources. Ficin protease enzyme procedure and *in situ* experiment were conducted in calves for a period of 12 weeks to find out the UDP content of soyabean meal (SBM) and extruded SBM by Maiga *et al.* (1994) and they found that extruded SBM exhibited 36.7% UDP over 31.3% observed in soyabean meal. Bunting *et al.* (1996) reported that diets with roasted soyabean contained more lysine (0.59% vs 0.51%) and marginally more methionine (0.2% vs 0.18%) and total essential amino acids (5.6% Vs 5.4%) when compared to the mixture containing feather meal and blood meal. Wang and Feng (1996) reported the nitrogen degradability of soyabean meal in the rumen as 91 per cent.

The presence of antinutritional factors such as protease inhibitors, hemagglutinins, safonins, goitrogenic factors, rachitogenic factors, allergenic factors and metal chelating factors in raw soyabeans affect the performance of animal when fed untreated (Loon, 1996). Comert and Sayan (2000) evaluated RDP and UDP contents of various ruminant feeds and observed that UDP content was highest in soyabean meal among maize, wheat scruf, lucerne and cotton seed.

2.2 Protection of feed proteins

The discovery made by McDonald (1948) that the soluble dietary proteins are extensively degraded to ammonia in the rumen led to the concept of protection of proteins against microbial degradation. The most promising approach seemed to be the modification of dietary proteins by formaldehyde treatment (Ferguson *et al.*, 1967; Faichney, 1971; Gupta and Gupta, 1984).

There are several alternatives for reducing or preventing the degradation of proteins in the rumen so that they would pass to the lower gut for subsequent digestion viz., closure of reticulo-rumen groove (Orskov and Fraser, 1969), by feeding liquid diets or by feeding dietary proteins subjected to heat treatment (Chalmers *et al.*, 1954; Chalmers *et al.*, 1964; Bhargava *et al.*, 1975; Sengar and Mudgal, 1982a), tannic acid treatment (Leroy *et al.*, 1965; Delort-Laval and Zelter, 1968; Bhargava *et al.*, 1973) or formaldehyde treatment (Reis and Tunks, 1969). Chalupa (1975), in a review reported that certain chemical agents form reversible cross linkages with amino and amide groups which decrease solubility of protein at the pH of the rumen. Chemically-treated proteins subsequently are made available to the host by destruction of these linkages in the acidic condition of abomasum. Agents investigated include aldehydes, tanning materials and chemicals such as phosphonitrilic halides, polymerized unsaturated carborylic acids, halo-triazines, sulfonyl halides, acrolein acetals, hexamethylene tetramine and acetylenic esters. More research had been carried out on treatment with formaldehyde than with other chemical agents.

There are a number of processing methods for soyabean meal as suggested by Waltz and Stern (1987), which included heat and non-heating methods. The latter included treatment with aldehydes (such as formaldehyde and glutaraldehyde), bentonite, sodium hydroxide, tannins, alcohol, blood, fish hydrolysate, propionic acid and calcium lignosulfonate. Among these it was

found that expeller processing, calcium lignosulfonate and formaldehyde were the most effective methods in reducing ruminal protein degradation.

2.2.1 Protection of feed proteins by formaldehyde treatment

The formaldehyde leads to the formation of methylol group on the terminal ∝-amino group of protein chain and β-amino groups of lysine to form methylene bridges at pH 6.0, thereby decrease the solubility of proteins (Fraenkel-Conrat and Olcott, 1948), which make them resistant to microbial attack (Walker, 1964; Reis and Tunks, 1969). Peter *et al.* (1971) reported that treating the high quality proteins of soyabean meal with formaldehyde reduced the rumen solubility and was a potential method of decreasing their degradation in the rumen, allowing them to bypass the rumen and then to abomasum and lower digestive tract. Longlands (1971) also noticed decreased nitrogen digestibility when casein was subjected to formaldehyde treatment. Nishimuta *et al.* (1973) noted a significantly ($P<0.05$) lower digestibility coefficient when crude protein of soybeans was treated with formaldehyde than with heat and tannic acid treatments.

Formaldehyde treated proteins, when fed to sheep, there was an increase in the amount of bypass protein with an increase in wool growth (Ferguson, 1975) and weight gain of young ruminants (Spears *et al.*, 1979; Tamminga, 1979; Spears *et al.*, 1980) compared to feeding of untreated protein. Thomas *et al.* (1979) reported that the optimum formaldehyde level for feedlot performance was 0.8 g/100 g crude protein or 0.35 g/100 g meal. Chalupa

(1984) reported that treatment of a wide range of feedstuffs with formaldehyde substantially increased amount of leucine, isoleucine, valine, histidine, arginine and phenyl alanine absorbed from the small intestine.

Tiwari and Yadava (1989) pointed out that formaldehyde treated mustard cake had no adverse effect on volatile fatty acids production, *in vivo* rumen fermentation pattern and rumen microbial transaminase activities of adult male buffaloes and concluded that 1 g formaldehyde/100 g crude protein was sufficient for optimum protection of mustard cake protein from microbial degradation. Tiwari and Yadava (1990) also agreed that 1 g formaldehyde/100 g crude protein in mustard cake was sufficient to protect the proteins without affecting the nutrient utilization.

Garg (1998) opined that proteins were to be protected to raise the level of rumen undegradable protein (UDP) to about 70 per cent and accordingly the levels of either heat or formaldehyde were to be adjusted. Dutta and Agarwal (2000) on determining protein solubility by *in vitro* method in McDougall'o artificial saliva found that heat (150°C, 4h) and formaldehyde treatments were most effective for decreasing effective degradation of crude protein irrespective of supplement

2.2.2 Protection of soyabean meal by formaldehyde treatment

Treatment of proteins including soyabean meal with formaldehyde had been shown to increase the quantity of protein entering the lower digestive tract of ruminants (MacRae *et al.*, 1972; Nishimuta *et al.*, 1974; Faichney and

White, 1977). Thomas *et al.* (1979) on evaluating protective agents applied to soyabean meal and fed to young cattle by feedlot trials implied that the most responsive formaldehyde treatment level was 0.8 per cent of the soyabean meal protein and reported that cattle receiving this level gained weight by 35 per cent more rapidly than the negative control cattle receiving untreated soyabean meal. The overall performance of the cattle receiving this level was nearly as good as that of the cattle receiving the positive control diet which contained three times as much as soyabean meal without treatment. ?

Phillips (1981) on *in vitro* digestion of formaldehyde treated soyabean meal suggested that optimum formaldehyde level for maximizing nitrogen utilization in ruminants was between 0.35 to 0.5 g/100 g soyabean meal.

Srivastava and Mani (1991) reported that formaldehyde treated soyabean meal tended to have more crude protein than untreated meal (24.3% vs 23.8%) and opined that feeding formaldehyde treated soyabean meal reduced the fermentative activity and increased the nitrogen utilization in adult Murrah buffaloes. Bhagwat and Srivastava (1993) reported that the treatment of soyabean cake with heat, formaldehyde or tannic acid lead to higher absorption of amino acids than when protein hydrolysate of soyabean cake was untreated, due to changes in physico-chemical properties of proteins and they concluded that formaldehyde treatment had an edge over other treatments. Giri and Dass (1993) recorded an increased crude protein content of formaldehyde treated mustard cake over untreated cake. Dutta and Agarwal (1995) reported that the solubility of soyabean meal decreased significantly ($P<0.01$) and maximum

over what ?

protection was provided by the heat treatment at 150°C for 4 h followed by formaldehyde 1% treatment.

Formaldehyde treatment of soyabean meal in ruminants at the rate of 0.5 per cent (0.5 g/100 g meal) decreased the soluble fraction to near zero and Cerda *et al.* (1998) concluded that 0.5 per cent level overprotected the soyabean protein, reducing rumen degradation and digestion, increasing fecal and urine nitrogen concentrations and decreasing nitrogen retention. Sarcicek (1998) reported that formaldehyde treatment of soyabean meal was a useful method for increasing the digestibility of feeds by reducing the rumen degradability of proteins.

2.3 Effects of rumen undegradable proteins (UDP)

2.3.1 On milk yield

Satter *et al.* (1970) observed an increase of 0.16 per cent fat content of milk in lactating lambs and cows by feeding formaldehyde treated soyabean meal than the untreated meal. Verite and Journet (1977) observed that there were increase in milk, milk protein and milk fat yields in dairy cattle by feeding formaldehyde treated soyabean and rapeseed meals. Sengar and Mudgal (1982b) reported that there was an increase in the milk yield with highest efficiency in the conversion of dry matter, energy and protein in the milk of Beetal goats fed with formaldehyde treated groundnut cake among heat, tannic acid treated as well as untreated cakes. Rees *et al.* (1983) also obtained significant increase in milk yield and total solids yield in lactating cows on feeding formaldehyde treated soyabean meal. Crawford and Hoover (1984)

was of the opinion that efficiency of milk production was higher by feeding formaldehyde treated soyabean meal in dairy cows compared to untreated soyabean meal.

Chalupa (1984) reported that when there was an increase in dietary crude protein from 9-10 to 13-14 per cent based on corn and soyabean meal, there was a significant rise in milk yield with an increased protein content. Supplementing dairy cattle intake with undegradable protein had been shown to decrease milk fat and increase milk protein yield compared to intake with degradable protein supplementation (Oldham *et al.*, 1985; Rijpkema *et al.*, 1990). Chalupa *et al.* (1990) concluded that in order to have a high milk yield, if there was no fat supplement, crude protein in the ration must contain 37 per cent of rumen undegradable protein (UDP).

Komaragiri and Erdman (1992) reported that only with the incorporation of dietary undegradable protein, Holstein-Friesian cows exerted greater effects on milk yield with the advancement of lactation. Ramachandra and Sampath (1995) observed that those animals receiving higher level of UDP (55%) yielded 9.81 kg/animals/d milk as compared to 8.5 kg/animal/d milk yield in cows receiving lower UDP (26%) and they ascribed the reason being increased availability of digestible protein post-ruminally in these animals for the better response.

Srivastava and Mani (1995) reported that the quantity and quality of milk of crossbred cows in terms of increase in total solids and protein content were increased because of feeding formaldehyde treated soya protein. Triplett

et al. (1995) supported the view that milk yield was greater in post partum Brahman (*Bos indicus*) heifers and cows consuming the medium undegradable protein supplement (55.74%) than those consuming high (76.28%) and low (36.56%) undegradable protein supplements.

In contrast to above mentioned reports on effects of protected protein on milk yield, the following workers alienated such findings. Verite and Journet (1977) concluded that formaldehyde treatment of protein has failed to produce any significant improvements of lactation performance. Trials by Oldham *et al.* (1981) and Castle and Watson (1984) did not indicate any increase in yield of milk constituents when treated soya was given.

Crooker *et al.* (1983) presented that feeding soyabean meal treated with formaldehyde did not affect milk yield either during peak production (22 to 63 d) or when crude protein intake did not meet requirements (22-119 d) or during the complete experimental period (22 to 301 d). They suggested that the failure was resulted from (a) factors other than absorption of essential amino acids limited the productivity, (b) the protein was processed inadequately, (c) over protected and (d) microbial protein production in the rumen was increased. They conferred that the only significant effect of formaldehyde treatment of soyabean meal in dairy cows on performance was a reduction ($P<0.01$) of both crude protein percentage and crude protein yield in milk during 22-63 d and 22-119 d of lactation.

Crawford and Hoover (1984) reported that formaldehyde treatment reduced the solubility of soyabean meal from 22.7 to 2.9 per cent and such treatments did not increase milk production whereas, caused significant decrease of milk protein suggesting that the level of formaldehyde was higher and overprotection did not result in greater amino acid availability at the intestine. Voss *et al.* (1988) opined that resistant proteins may provide a more consistent decrease in milk production with alfalfa silage when compared to corn silage. Rogers *et al.* (1989) reported that their experiment failed to find any improvement in the efficiency of milk production when diets containing soyabean meal were supplemented with rumen protected methionine and lysine.

Hadjipanayiotou and Photiou (1995) studied the effect of level of formaldehyde treatment of soyabean meal in lactating Chios ewes which were in negative energy balance and concluded that there was no significant effect of either protein level or soyabean meal treatment on milk yield, fat corrected milk yield or milk composition.

2.3.2 On reproduction

Diets high in ruminally degradable intake protein (DIP) have been shown to be detrimental to reproduction (Canfield *et al.*, 1990) and to transferable embryo yield (Blanchard *et al.*, 1990) in dairy cows. However, protein supplements with a high potential for rumen escape (UIP) have been shown to improve reproductive traits when fed in excess of National Research Council recommendations (Wiley *et al.*, 1991).

Triplett *et al.* (1995) reported that post partum Brahman (*Bos indicus*) first calf heifers and dams receiving the medium undegradable protein (55.74%) supplement had the highest percentages of normal first oestrous cycle length, corpus luteal functions and first service conception rates when compared with those receiving the low undegradable protein (36.56%) supplement and the highest undegradable protein (76.28%).

Bharadwaj *et al.* (2000) concluded that partially replacing conventional dietary unprotected protein by bypass protein resulted in improved nitrogen utilization and marginal shorter service period.

2.3.3 On growth

Bassett *et al.* (1971) and Wright (1971) had reported that treatment of milk casein with formaldehyde resulted in an increased nitrogen retention, wool growth and muscle growth in various species of ruminant animals. Significantly increased growth rate in Muzzafaranagri weaned lambs, fed with formaldehyde treated groundnut cake had been reported by Bhargava and Ranjhan (1973). Increased growth rate in lambs on consuming formaldehyde treated soybean meal was also reported by Nimrick *et al.* (1972).

Spears *et al.* (1980) reported that formaldehyde treatment resulted in a linear increase in average daily gain of growing steers over the negative control diet of 0 per cent formaldehyde, during the entire 90 days experimental period. Treating dietary proteins with formaldehyde increased wool growth and

increased weight gain in young rapidly growing sheep as reported by Chalupa (1981).

Sengar and Mudgal (1982a) opined that maximum retention of nitrogen took place in crossbred male kids by feeding 1% formaldehyde treated groundnut cake. Hosmani and Srivastava (1984) found that gain in body weight was more in buffalo calves, which were offered formaldehyde treated soyabean in concentrate mix.

Gupta and Gupta (1984) found that increment in body weight of Karan Swiss calves was highest when they were fed with the ration having crude protein treated with formaldehyde @ 1 g% rather than with 1.5 g% formaldehyde treatment. Sampath and Sivaraman (1986) concluded that crossbred female calves of 4-6 months of age required 117 g of UDP/kg concentrate and 95 g of RDP/kg concentrate ration for better growth rate and a 45 per cent of RDP was sufficient to have optimum growth rate. Several reports are there in calves explaining about increased growth rate and feed efficiency as a result of increased dry matter intake, energy and protein by the incorporation of UDP sources at various levels viz., Upadhyaya and Gupta (1988) at 0 per cent, 33 per cent and 66 per cent formaldehyde treated groundnut cake and Smith *et al.* (1990) using different sources of bypass protein meal.

Giri and Dass (1993) recorded an adverse effect on growth rate of buffalo calves fed 15 per cent less crude protein than the requirement and a

more efficient utilization of bypass protein in the lower gastrointestinal tract could be observed on supplementation of feed with 1 per cent formaldehyde treated mustard cake. Zinn and Owens (1993) observed that daily gain in feedlot calves was higher with 2 per cent UDP added to the corn based growing diet and they concluded that the lowest level (2 per cent) increased the rate and economy of gain in growing steers.

Kumar and Walli (1994) reported that urea treated wheat straw supplemented with formaldehyde treated groundnut cake in crossbred calves of 8-10 months could improve the growth rate and feed efficiency. They also found that growth rate in calves was better when untreated straw was supplemented with formaldehyde treated groundnut cake. Maiga *et al.* (1994) reported that increased ruminally undegradable carbohydrate and undegradable protein increased the body weight gain in Holstein calves, which may be due to a greater dry matter intake. They also suggested that less ruminally degradable carbohydrate from corn with less degradable protein from extruded soyabean meal fed in pelleted form to young dairy calves probably were more palatable and they would also have provided more nitrogen and carbohydrate to the small intestine for digestion and absorption thereby, resulting in better body weight gains.

Triplett *et al.* (1995) reported that calves born to animals receiving the medium undegradable intake protein (55.74%) supplement gained more ($P<0.08$) than those calves of animals receiving either the low (36.56%) or high (76.28%) undegradable intake protein diets during a period from birth to

weaning. Bunting *et al.* (1996) found that during winter, Holstein male calves fed the high percentage of rumen undegradable protein had better body weight gain as well as body weight gain: dry matter intake and the values were similar to those of calves fed moderate undegradable protein during summer. Calves fed the high percentage of rumen undegradable protein during summer had higher body weight gain (1.39 vs 1.19 kg/d) and body weight gain: dry matter intake (0.27 vs 0.23) when compared to calves fed with moderate undegradable protein. They concluded that an increased percentage of undegradable protein in the diet improved the growth of heat-stressed calves and the source of crude protein might be important during heat stress.

Mehta and Srivastava (2001) reported that the increase in efficiency of nitrogen utilization and better growth rate by feeding formaldehyde treated barley in crossbred calves of 3-5 months could be due to formaldehyde treatment of barley portion of concentrate mixture.

2.4 Effect of rumen undegradable proteins (UDP) on physiological parameters

The available literatures with respect to the effect of dietary protected proteins on routine haematological parameters were very scanty. Only a handful of references are available on biochemical and certain hormonal parameters which have been reviewed in this section.

2.4.1 Haematological parameters

Pande and Shukla (1979) reported that there was non-significant differences in the levels of haemoglobin concentration, total erythrocyte count and total leucocyte count of Surti buffalo calves when fed with different levels of digestible crude protein and the results were in consonance with the earlier works by several authors including Patel *et al.* (1972) and Hussain *et al.* (1975).

2.4.2 Biochemical parameters

2.4.2a Serum protein profile

Payne *et al.* (1970) found hyper albuminaemia in herds reared on high intake of crude protein with increased blood urea nitrogen. Nishimuta *et al.* (1974) reported that casein protected by formaldehyde had the same metabolic value as casein infused into the abomasum and it increased plasma concentrations of amino acids. Treacher *et al.* (1976) and Jordan and Swanson (1979) reported that percentage crude protein in the ration of high producing dairy cows did not affect total protein or albumin in the serum.

Rekwot *et al.* (1989) reported that animals on high protein diets (14.45% crude protein) had significantly ($P<0.05$) higher serum total protein, albumin, α-2 globulin, γ-globulin and total globulin than those on low protein ration (8.5% crude protein). Tiwari and Yadava (1990) recorded a significantly higher plasma protein concentrations in buffaloes fed with formaldehyde treated mustard cake and levels of inclusion as well as formaldehyde did not influence the plasma protein concentration.

Zinn and Owens (1993) reported that post ruminal amino acid flow in feedlot calves could be directly altered by supplementing undegradable protein. They also concluded in growing steers that arginine, histidine, lysine, methionine and phenylalanine seemed to be the most limiting amino acids in a corn based growing diet and bypass protein at the lowest level (2%) increased the rate and efficiency of body weight gain. Swenson and Reece (1996) reported that intake of protein beyond daily needs resulted in the formation of increased urea and disclosed that carbohydrates provided a greater stimulus for insulin secretion, which promotes protein anabolism. Kaneko *et al.* (1997) reported a direct correlation between albumin turn-over and body size.

Pachauri *et al.* (1999) while carrying out a growth trial in female crossbred calves fed sorghum silage based rations found that except glucose content, serum protein and urea nitrogen were significantly ($p<0.05$) influenced in a positive trend by the dietary treatments.

2.4.2b Serum lipid profile

Hutjens and Schultz (1971) reported that the levels of plasma triglycerides, cholesterol (free) and free fatty acids reduced in lactating goats supplemented with formaldehyde treated soybeans. It was concluded that free cholesterol was significantly ($P<0.05$) higher for soybean treatments. They also concluded that the formaldehyde treatment did not increase the amount or degree of unsaturation of lipids in plasma or milk. Park (1985) on evaluating the effects of both low (12%) or high (25%) dietary crude protein on growth

and serum metabolites in male Holstein calves found that there was a decrease in the concentration of total serum cholesterol and an increase in serum urea nitrogen without any variation in total serum protein and glucose in high protein diet fed calves. He added that a reciprocal relationship existed between the dietary protein level and plasma cholesterol concentration. Bunting *et al.* (1996) reported that calves fed with rumen undegradable protein as roasted soybeans tended to have a smaller percentage decrease in concentrations of non-esterified fatty acids (NEFA) relative to feeding.

2.4.2c Blood glucose level

Grubic (1991) reported that rumen undegradable protein (UDP) level did not influence blood glucose, blood urea and total protein levels. Palmquist *et al.* (1993) reported that blood glucose concentrations of cows were not influenced by the ration high in fat and undegradable protein. Bunting *et al.* (1996) reported that concentration of glucose in plasma was greater both before and at 3 h after feeding supplemental undegradable protein and the percentage increase as a result of feeding was greater when calves were sampled in winter, compared to summer. Swenson and Reece (1996) reported that under post-absorptive conditions, blood glucose level varied considerably (between 60-80 mg/dl) and the variability was often related to nutritive state.

2.4.2d Serum urea nitrogen / blood urea nitrogen (BUN)

Pangborn (1978) observed that levels of urea nitrogen in plasma increased significantly from 9.08 mg/dl to 18.25 mg/dl when crude protein

nitrogen level. Sharma *et al.* (1972) reported a significant decrease in the plasma urea nitrogen in calves fed formaldehyde treated rapeseed meal. Spears *et al.* (1980) observed a significant linear and quadratic decrease in urea nitrogen concentrations in serum when increasing levels of formaldehyde treated soybean meal was fed in growing steers. Tiwari and Yadva (1990) observed that the concentration of plasma urea nitrogen of buffalo calves differed significantly ($P<0.05$) with lowest value in 2 per cent formaldehyde treated mustard cake group.

Griffin *et al.* (1993) made an assessment of protein quality of heat treated soyabean products and reported that calves fed with heat damaged soybeans tended ($P<0.12$) to have lower ruminal ammonia nitrogen concentrations and correspondingly, had lower ($p<0.01$) serum urea concentration of 4.1 mM whereas calves fed the other soybean products viz., soybean meal, roasted soybeans and raw soybeans exhibited serum urea concentrations of 5.8 mM, 6.1 mM and 6.3 mM respectively. Chen *et al.* (2002) found that Holstein cows fed with undegradable intake protein of heat extruded dietary soyabean meal and protected fat supplement showed a significant decrease in the plasma urea nitrogen concentration ($P<0.01$) without any significant influence in the plasma cholesterol concentration. Similar results with regard to plasma urea concentrations were also reported by Broderick *et al.* (1993) and Tomlinson *et al.* (1994).

2.4.3 Certain hormonal parameters

Diet influences the hormone secretion in both non ruminants (Munro, 1964) and ruminants (Bassett *et al.*, 1971; Trenkle, 1970) especially growth hormone (GH) and insulin. The concentrations of GH and insulin were increased in cattle, sheep and goats when amino acids either individually (arginine) or in mixtures (casein hydrolyses) were intravenously infused.

Faichney and Weston (1971) found that feeding of formaldehyde treated casein in sheep resulted in an increased plasma concentration of insulin and amino nitrogen with a decrease in plasma urea. Hoch (1974) reported that thyroid hormones stimulated the basic metabolic rate via the metabolism of carbohydrate, lipids and proteins. Oldham *et al.* (1982) observed a significant increase in plasma concentration of growth hormone when formaldehyde treated casein or treated soya were fed orally in calves. Waghorn *et al.* (1987) reported that plasma concentration of insulin was increased in sheep fed with 22 per cent crude protein diets than those fed with 12 per cent crude protein diets.

Barry *et al.* (1982) reported that directly infusing sodium caseinate at different rates viz., 44 g/d and 300 g/d and methionine @ 0.5 g/d into abomasum resulted in a significant increase ($p<0.03$) of insulin concentration in growing lambs on ryegrass pasture. Guerino *et al.* (1991) opined that increased insulin secretion would be utilized for protein anabolism and triglycerides synthesis. They also demonstrated a positive relationship between amino acid

absorption and pancreatic insulin secretion. Ragland *et al.* (1998) reported that growing ruminants consuming high protein grass forages appeared to be benefited from supplemental protein through provision of greater duodenal amino acid flow and a subsequent increase in anabolic hormone levels.

Barash *et al.*(1998) reported that the plasma concentrations of key hormones as thyroxine (T_4) and insulin and insulin-like growth factor (IGF) which control growth and metabolism were shown to be effected by feed restriction and concluded that plasma concentration of total T_4 was found to be an indicator of energy balance, body weight gain and protein deposition The latter part had extensively been studied by several authors including Bunting *et al.* (1996) and Alshaikh *et al.* (1997). Neeru *et al.* (2001) in a study in growing male Muzzafaranagri lambs found that there was a decreasing trend in the T_4 concentration from third month to 16 months of age and concluded that thyroid hormones are important in the regulation of early growth in sheep.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Experimental animals

The study was conducted in a group of female crossbred calves of similar age (6 months) and weight (50 ± 5 kg) of the University Livestock Farm, College of Veterinary and Animal Sciences, Mannuthy for a period of 90 days (6 to 9 months age). The calves were divided into two groups viz., group I, (G I - control) and group II, (G II - experimental) comprising six animals in each group. The animals were housed in a well-ventilated shed and reared under the standard managemental conditions. During the experimental period, calves were treated for internal and external parasitic infections.

3.2 Feeding schedule

The quantity of concentrate ration to be given (1.5 kg/animal/day) to the animals for a period of 90 days was calculated according to ICAR Standards (1985) and Package of Practices Recommendations, Kerala Agricultural University (2001) and 1/3rd quantity (0.5 kg) thus arrived at, was replaced by raw soyabean meal (solvent extracted) in G I (control) and formaldehyde (H-CHO) treated soyabean meal in G II (experimental).

Weighed quantity (0.5 kg) of raw soyabean meal was offered to each animal of G I once daily at 07:00 h and immediately after its consumption, the balance (1 kg) of concentrate as pellets was given. Similarly, weighed quantity (0.5 kg) of formaldehyde treated soyabean meal was offered to each animal of G II once daily at 07:00 h and immediately after its consumption, the balance

(1 kg) of concentrate as pellets was provided. The composition and crude protein content of concentrate ration fed to the calves of both groups are given in tables 1a and 1b. Roughage in the form of cultivated grass was also provided *ad lib* both in the morning (08:00 h) and evening (15:00 h). All the animals were provided with drinking water *ad lib*. Records of daily feed consumption were maintained for each animal.

Crude protein (CP) content of soyabean meal as well as rations with and without soyabean meal were determined as per standard methods suggested by AOAC, 1990 (Microkjeldhal technique).

3.3 Formaldehyde treatment of soyabean meal

The ground soyabean meal was spread in thin layers in enameled trays for treating with formaldehyde at the rate of 1.0 g per 100 g crude protein (Tiwari and Yadava, 1988). As the CP content of soyabean meal was found to be 45 per cent, an amount of 2.5 ml H-CHO solution (w/v 37-41%) was required for 100 g CP. This volume of H-CHO solution was diluted thrice with water and sprayed over the soyabean meal and mixed immediately manually by hand for about 15 min. Thereafter, the meal was packed and sealed air-tight in polythene bags allowing the formaline fumes to equilibrate with ground soyabean meal. It was kept as such for one week for proper reaction of formaldehyde with proteins. Polythene bags were then opened and the treated products were again dried in air for a day to allow excess fumes to evaporate

and thereby palatability problems were avoided. Later on, they were packed as ½ kg packets, ready to feed to each animal.

3.4 Recording of body weight gain of animals

Body weight of all the animals of G I and G II were recorded fortnightly during the entire period of the study of 90 days and one month thereafter using the equation of $W = L \times G^2 / 300$ (where W = weight in pounds, L = length from point of shoulder to point of buttock in inches and G = girth of the body behind withers in inches) as prescribed by Ensminger *et al.* (1990).

3.5 Collection of blood samples

Blood samples were collected from all animals of both G I and G II by jugular vein puncture at the start of experiment (180 d), thereafter on every 14 days and one month after the completion of experimental period (300 d) at a specific time before feeding (06.30 h). Three ml of whole blood was collected in clean, dried, labeled vials containing sodium salt of ethylene diamine tetra-acetic acid (EDTA @ 1.5 mg/ml of blood) as anticoagulant and was used for the estimation of various haematological parameters. Another 15 ml of blood was collected in labeled test tubes and serum was separated by centrifuging at 3000 rpm for 20 min. Clear serum was aliquoted and transferred to labeled serum vials and stored at -20°C till further analysis.

3.6 Estimation of haematological parameters

3.6.1 Haemoglobin (Hb) concentration: Haemoglobin (Hb) concentration was estimated by Cyanmethaemoglobin method as suggested by Zijlstri (1960), using Haemocheck Kit (M/s Agappe Diagnostics, India).

3.6.2 Total erythrocyte (RBC) count, total leucocyte (WBC) count and volume of packed red blood corpuscles (VPRC) were determined on the day of blood collection as per standard procedures (Jain, 1986).

3.6.3 Erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulas (Swenson and Reece, 1996).

3.7 Estimation of biochemical parameters

3.7.1 Protein profile

3.7.1.1 Concentration of total protein: Total protein content of serum was estimated by Biuret method, as suggested by Henry *et al.* (1957) using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai).

3.7.1.2 Concentration of albumin : Concentration of serum albumin was estimated by Doumas method, as described by Doumas *et al.* (1971) using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai).

3.7.1.3 Concentration of globulin and A:G ratio

The serum globulin content was determined by substracting serum albumin level from the total serum protein content and subsequently, A:G ratio was calculated.

3.7.2 Lipid profile

3.7.2.1 Concentration of total lipids: Concentration of serum total lipids was estimated by Phosphovainilline method, as described by Chabrol (1961) using Labkit® Kit (M/s Labkit, Spain).

3.7.2.2 Concentration of cholesterol: The concentration of total serum cholesterol was estimated by Cholesterol Phenol Aminoantipyrine (CHOD-PAP) method, as suggested by Richmond (1973) using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai).

3.7.2.3 Concentration of triglyceride: Concentration of serum triglyceride was estimated by a method suggested by Schettler and Nussel (1975) using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai).

3.7.2.4 Concentration of non-esterified fatty acids (NEFA): Serum NEFA concentration was estimated by a method suggested by Faholt *et al.* (1973) using copper soap formation.

Principle

Serum is extracted with a chloroform-heptane-methanol mixture in the presence of a phosphate buffer to eliminate interference from phospholipids and

the extract is shaken with a high density copper reagent at pH 8.1. The copper soaps remain in the upper organic layer from which an aliquot is removed and the copper content determined colorimetrically with diphenyl carbazide.

Reagents required

1. Extraction solvent containing chloroform, heptane and methanol (5:5:1) was prepared.
2. Phosphate buffer (pH 6.4, 33 mmol/l): Two volumes of potassium dihydrogen phosphate (4.539 g/l) were mixed with one volume of disodium hydrogen phosphate dihydrate (5.938 g/l) to prepare the buffer.
3. Stock copper solution (500 mmol/l): 12.07 g of copper nitrate trihydrate ($CuCNO_3)_2.3H_2O$) was dissolved in distilled water and the volume was made to 100 ml with distilled water.
4. Triethanolamine solution (1 mol/l): 10 ml of triethanolamine was diluted to 100 ml with distilled water to prepare 1 mol/l solution.
5. Sodium hydroxide solution (1 mol/l): 4 g of sodium hydroxide was dissolved in distilled water and the volume was made to 100 ml using distilled water.
6. Copper reagent: 10 ml of stock copper solution, 10 ml of triethanolamine solution and 6 ml of sodium hydroxide solution were mixed and diluted to 100 ml with distilled water to which, 33 g of sodium chloride was added and the pH was adjusted to 8.1, using 1 mol/l sodium hydroxide solution.

7. 1, 5 Diphenylcarbazide solution (4 g/l in ethanol): 40 mg of Diphenylcarbazide was dissolved in 10 ml ethanol to which 0.1 ml of triethanolamine solution was added (prepared immediately before experiment).

8. Stock standard palmitic acid solution (2 mmol/l): 51.2 mg of palmitic acid was dissolved in the extraction solvent and the volume was made to 100 ml using extraction solvent. This solution was stored in a tightly stoppered container.

9. Working standard palmitic acid solution: 5 ml of stock standard palmitic acid solution was diluted to 20 ml with extraction solvent to give a solution containing 500 µmol/l (prepared freshly).

Procedure

1. To 50 µl serum in a suitable stoppered centrifuge tube, 1 ml phosphate buffer and 6 ml extraction solvent were added. At the same time, 50 µl working standard palmitic acid solution was also prepared in another centrifuge tube in the same fashion.

2. The tubes were shaken vigorously for 90 sec, left undisturbed for 15 min and then, centrifuged at 4000 rpm for 10 min.

3. The buffer was carefully removed by suction and 5 ml of extraction solvent settled at the bottom of the tubes, was transferred to a similar dry centrifuge tube to which 2 ml of copper reagent was added.

4. The tubes were shaken vigorously for 5 min and then, centrifuged at 3000 rpm for 5 min.

5. 3 ml of the upper layer was transferred to a tube containing 0.5 ml phenyl carbazide solution and mixed carefully.

6. The reading was taken after 15 min at 550 nm in a spectrophotometer.

Calculation

$$\text{Serum NEFA } (\mu\text{mol/l}) = 500 \times \frac{\text{Reading of unknown}}{\text{Reading of standard}}$$

3.7.3 Blood glucose level (BGL): Blood glucose level (BGL) was estimated by glucose oxidase perioxidase method (GOD-POD method), immediately after blood collection, as suggested by Mayne (1994b) using Ecoline ® (M/s E. Merck (India) Limited, Mumbai).

3.7.4 Serum urea nitrogen content : Serum urea nitrogen content was estimated by Diacetyl Monoxime (DAM) method, as suggested by Mayne (1994a) using Urea Kit® (M/s Dr. Reddy's Laboratories, Hyderabad).

3.7.5 Serum creatinine : Serum creatinine was estimated by kinetic method of Jaffe reaction without deproteinisation as described by Helger *et al.* (1974) using Merckotes® Kit (M/s E. Merck (India) Limited, Mumbai).

3.7.6 Serum bilirubin level: Serum bilirubin level was estimated by the method of Jendrassik and Grof (1938) using Merckotest® Kit (M/s E. Merck (India) Limited, Mumbai).

3.8 Hormonal profile

3.8.1 Thyroxine (T_4): Serum T_4 concentration was estimated using the gamma coat T_4 radioimmunoassay commercial kit (M/s Diasorin, Minnesota, USA).

3.8.2 Triiodothyronine (T_3) and T_4:T_3 ratio: Serum T_3 concentration was estimated using the gamma coat T_3 radio immunoassay commercial kit (M/s Diasorin, Minnesota, USA). Later on, T_4:T_3 ratios were calculated.

3.8.3 Insulin: Serum insulin concentration was estimated using radio immuno assay commercial kit (INSIK-5®) (M/s Diasorin, Saluggia, Italy).

Inter and intra assay coefficients of variation for the determination of above mentioned three hormones were recorded and it was found to be less than 10 per cent.

3.9 Fractionation of serum protein by Agarose Gel Electrophoresis

The serum samples were subjected to get electrophoretic fractionation using 1% agarose in Tris-HCl buffer (pH 8.6) for constructing the gel. After subjecting for 2 hrs electrophoretic run (30 mA, 150 V), the gel was fixed in methanol (30 min) and air dried (80°C, overnight). The slides were stained with 1% amido black (30 min) and destained using 5% acetic acid till the protein bands were clearly visible (Talwar, 1983).

3.10 Statistical analysis

The data recorded were statistically analysed by employing student *t* test for comparing both within the group and between groups (Snedecor and Cochran, 1989).

Results

4. RESULTS

4.1 Composition and crude protein content of the ration

The composition and crude protein content of the rations fed to the crossbred calves is presented in tables 1a and 1b. The formaldehyde treated soyabean meal had 2% crude protein (47%) more than the untreated one (45%). Hence, the concentrate mixture fed to the animals of experimental (G II) group during the experimental period (180-270 d) had higher crude protein content of 30.33 per cent compared to 29.67 per cent crude protein content of concentrate ration fed to animals of control (G I) group. However, the crude protein content of the standard concentrate mixture offered to the calves in the farm during the period of 271-300 d was 22 per cent.

4.2 Body weight gain of animals

The body weights of female crossbred calves fed the concentrate ration supplemented with untreated soyabean meal and formaldehyde treated soyabean meal are presented in table 2 and fig.1. The initial body weight in animals of control group (G I) and that of experimental group (G II) were 50.645 kg and 47.205 kg respectively. Similarly, the mean body weight of animals of G I at the end of the trial (at 270th day) was more (80.115 kg) than that of animals of G II (78.950 kg). However, the body weight gain per animal during the experimental period in animals of G II was more (31.745 kg) than that of animals of G I (29.470 kg).

Table 1a. Composition of the concentrate ration* fed to the crossbred calves from 180 to 300 days of age

Sl. No.	Ingredients in kg	180-270 days		271-300 days
		Group I	Group II	Group I and II
1.	Groundnut cake (GNC)	20.0	20.0	30.0
2.	Coconut oil cake (COK)	6.7	6.7	10.0
3.	Rice bran	20.0	20.0	30.0
4.	Yellow maize	18.0	18.0	27.0
5.	Soyabean meal	35.3	Nil	Nil
6.	Formaldehyde treated soyabean meal	Nil	35.3	Nil
	Total	100	100	100
7.	Mineral mixture*	2.0	2.0	2.0
8.	Salt (Sodium chloride)	1.0	1.0	1.0

(*Source: Kerala Feeds, Thrissur)

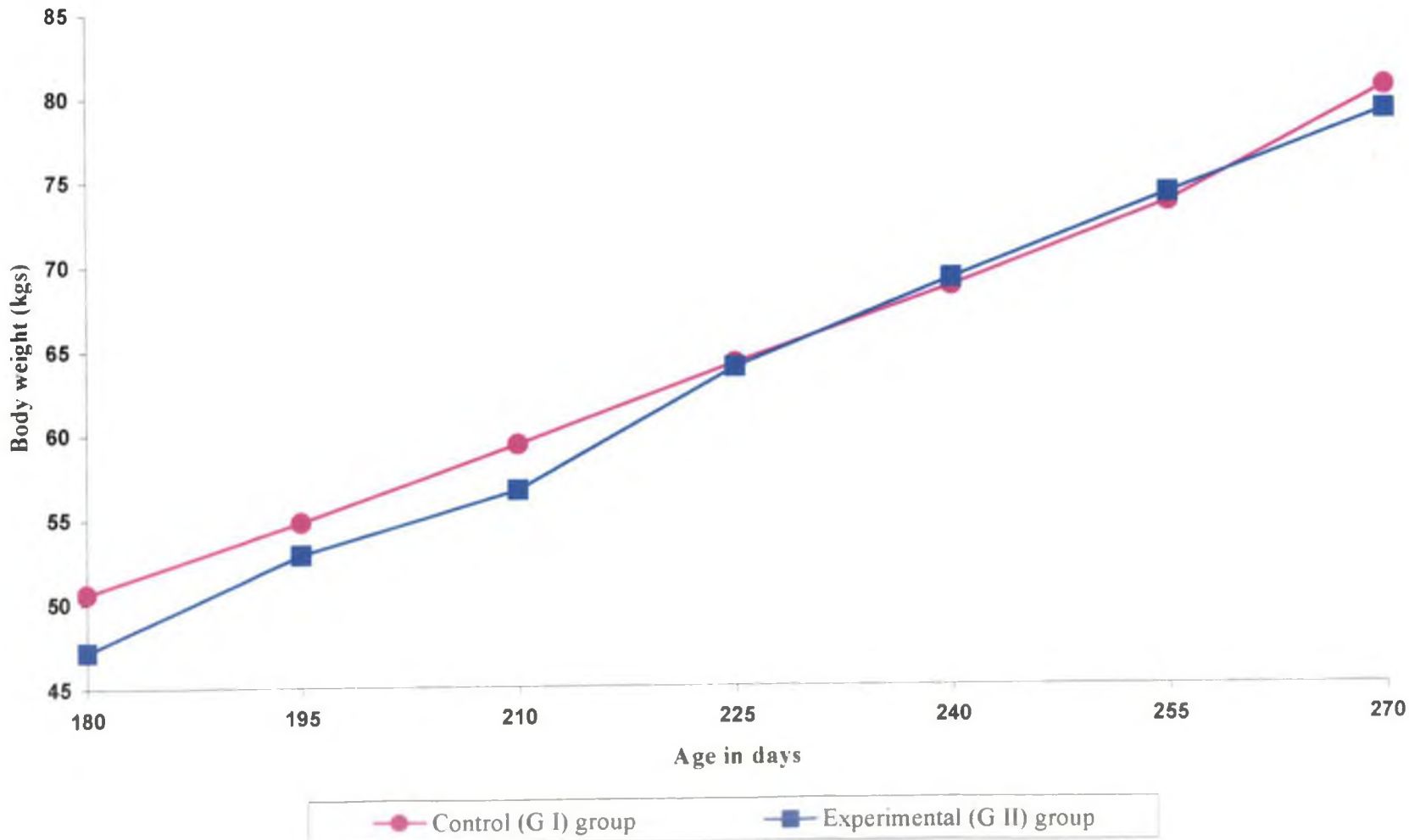
Table 1b. Crude protein content of the ration fed to the crossbred calves

Sl. No.	Ingredients / feeds	Crude protein (%)
1.	Soyabean meal	45.00
2.	Formaldehyde treated soyabean meal	47.00
3.	Concentrate mixture (271-300 days)	22.00
4.	Concentrate mixture for Group I (180-270 days)	29.67
5.	Concentrate mixture for Group II (180-270 days)	30.33

Table 2. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on body weight of crossbred calves (n=6)

Group	Animal number	Body weight (kg) before the trial (180 d)	Body weight (kg) at the end of trial (270 d)	Body weight gain (kg)	Daily weight gain per animal (g)
GROUP I	114	58.60	92.80	34.20	379.96
	115	46.22	81.33	35.11	390.19
	125	65.31	99.49	34.18	379.81
	130	48.34	74.39	26.05	289.47
	143	39.10	58.98	19.88	220.93
	146	46.30	73.70	27.40	304.43
Mean		**50.65 ± 12.06**	**80.12 ± 14.01**	**29.47 ± 9.12**	**327.46 ± 6.45**
GROUP II	132	44.11	73.15	29.04	322.64
	134	46.30	83.28	36.98	410.94
	135	39.02	72.42	33.40	371.16
	139	55.88	86.01	30.13	334.82
	140	53.48	81.33	27.85	309.47
	141	44.44	77.51	33.07	367.36
Mean		**47.21 ± 13.14**	**78.95 ± 12.06**	**31.74 ± 10.01**	**352.73 ± 4.56**

Fig.1. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on body weight of crossbred calves



The daily weight gain per animal of G II was higher (352.731 g) than that of animals of G I (327.460 g). The weight gain of animals of G II tended to increase steeply from 210 d of experiment (fig.1) when compared to control animals and followed the same trend till 255th d of trial, where the body weights of animals of G I and G II were 73.630 kg and 74.151 kg respectively (fig.1). This period represents the take-over advantage period of animals of experimental group over control animals.

4.3 Haematological parameters

The effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on certain haematological parameters such as, haemoglobin (Hb) concentration, total erythrocyte (RBC) count, total leucocyte (WBC) count and volume of packed red blood corpuscles (VPRC) are given in tables 3a, 3b and fig.2 and 3 and the effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on erythrocyte indices viz., mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are shown in tables 3c, 3d and fig.4.

4.3.1 Haemoglobin (Hb) concentration

There was an increasing trend in the concentration from 180 d to 270 d of study period in animals of both control (G I) and experimental (G II) groups.

The Hb concentration of animals of G I reached the highest value of 8.21 ± 1.90 g/dl at the end of the study period (270 d) when compared to the Hb

value of 7.25 ± 0.57 g/dl at the start of the experiment (180 d) vide table 3a. The Hb concentration came to the baseline value of 7.17 ± 1.17 g/dl, one month after the end of the trial (300 d) vide table 3b.

Similarly, the Hb concentration of animals of G II exhibited a continuous increasing trend and reached the peak value of 8.29 ± 0.62 g/dl on 270 d (table 3a), which was significantly ($P<0.05$) higher than 7.17 ± 0.82 g/dl on 180 d (table 3b). As in case of animals of G I, the Hb concentration of animals of G II also reached the baseline value of 7.17 ± 0.92 g/dl, one month after the end of trial (table 3b).

The comparison between animals of G I and G II carried out employing student *t* test revealed that there was no significant variation in the Hb concentration (table 3a).

4.3.2 Total erythrocyte (RBC) count

Calves of both control (G I) and experimental (G II) groups revealed an upstream trend in total RBC count during the entire period of study from 180 d to 270 d (table 3a).

In animals of G I, the total RBC count reached higher values from 255 d onwards and attained the highest value (7.84 ± 1.14 millions/µl) at the end of the trial (270 d) vide table 3a, when compared to the total RBC count (6.99 ± 1.46 millions/µl) at the start of the trial (180 d). One month after the end of the study (300 d), the total RBC count was lowered to 7.52 ± 1.98 millions/µl (table 3b).

Animals of G II followed the same tendency as that of animals of G I with respect to total RBC count and reached the highest value of 7.87 ± 1.17 millions/µl on 270 d from the value of 7.09 ± 1.81 millions/µl at the start of the trial (180 d) vide table 3a. One month after the end of study period (300 d), the total RBC count decreased to a new baseline value of 7.19 ± 1.61 millions/µl, showing a sudden decreasing trend (table 3b).

All comparisons between animals of G I and G II as well as within group revealed non-significant differences with respect to total RBC count (tables 3a and 3b).

4.3.3 Total leucocyte (WBC) count

A persistently increasing tendency was observed in total WBC count of animals of both control (G I) and experimental (G II) groups during the entire study period (180 d to 270 d).

The animals of G I showed a total WBC count of 10,925 ± 3,914 per µl at the initial period of experiment (180 d) and started increasing from then onwards to attain the highest value of 13,767 ± 3,927 per µl at the end of the trial (270 d) vide table 3a. The total WBC count then moderately decreased to reach the value of 12,342 ± 2,823 per µl, one month after the end of the trial (300 d) vide table 3b.

The total WBC count of calves of G II pursued a similar trend as that of animals of G I (table 3a), which reached the highest value of 14,058 ± 1,935 per µl on 270 d that was significantly ($P<0.05$) higher when compared to the

starting value of 10,708 ± 2,560 per µl on 180 d (table 3b). As in animals of G I, a moderate decrease of total WBC count was observed in animals of G II to attain a value of 12,700 ± 2,539 per µl on 300 d (table 3b).

No significant differences in total WBC count was revealed on comparison between animals of G I and G II groups employing student *t* test (table 3a).

4.3.4 Volume of packed red blood corpuscles (VPRC)

Similar to all the above three haematological parameters, the VPRC of animals of control (G I) and experimental (G II) groups pursued a definite upstream path.

The VPRC of animals of G I exhibited an increasing trend to reach the highest value of 31.17 ± 4.58 per cent at the end of the trial (270 d) vide table 3a, which was significantly ($P<0.05$) higher compared to the value of 25.67 ± 3.39 per cent (table 3b) at the start of the trial (180 d). Then, the VPRC declined to the baseline normal value of 25.50 ± 3.39 per cent (table 3b) on 300 d, i.e., one month after end of the trial.

The trend noticed in animals of G I was repeated in animals of G II, which showed the highest VPRC value of 29.00 ± 2.53 per cent on 270 d (table 3a), which was significantly ($P<0.05$) higher in comparison to the 180 d value of 24.50 ± 2.26 per cent (table 3b). The decline of VPRC in animals of G II group was moderate to reach a value of 26.17 ± 3.66 per cent (table 3b) on 300 d.

Table 3a. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on the haematological parameters of crossbred calves (n=6)

Age in days	Haemoglobin (g/dl)			Total erythrocyte count (millions/µl)			Total leucocyte count (numbers/µl)			Volume of packed red blood corpuscles (%)		
	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value
180	7.25 ± 0.57	7.17 ± 0.82	0.21^{NS}	6.99 ± 1.46	7.09 ± 1.81	0.11^{NS}	10,925 ± 3,914	10,708 ± 2,560	0.11^{NS}	25.67 ± 3.39	24.50 ± 2.26	0.70^{NS}
195	7.47 ± 0.79	7.21 ± 0.53	0.66^{NS}	7.25 ± 1.65	7.10 ± 1.40	0.17^{NS}	11,400 ± 2,645	11,425 ± 3,299	0.01^{NS}	27.00 ± 3.46	25.83 ± 1.83	0.73^{NS}
210	7.22 ± 0.85	7.24 ± 1.18	0.04^{NS}	7.31 ± 1.63	7.36 ± 1.52	0.05^{NS}	12,000 ± 2,056	12,185 ± 3,452	0.11^{NS}	29.00 ± 4.82	26.00 ± 3.29	1.26^{NS}
225	7.79 ± 1.36	7.67 ± 1.81	0.14^{NS}	7.55 ± 2.38	7.52 ± 1.61	0.64^{NS}	14,742 ± 3,123	13,217 ± 1,910	1.02^{NS}	30.33 ± 6.71	26.17 ± 2.93	1.39^{NS}
240	8.08 ± 2.22	8.17 ± 0.68	0.09^{NS}	7.62 ± 0.96	7.80 ± 1.52	0.25^{NS}	13,660 ± 3,101	13,800 ± 2,524	0.09^{NS}	30.52 ± 4.33	26.67 ± 2.88	1.82^{NS}
255	8.17 ± 1.97	8.21 ± 0.91	0.05^{NS}	7.83 ± 0.81	7.82 ± 0.75	0.02^{NS}	13,700 ± 1,040	12,800 ± 1,778	1.07^{NS}	30.58 ± 3.06	27.50 ± 2.07	2.05^{NS}
270	8.21 ± 1.90	8.29 ± 0.62	0.10^{NS}	7.84 ± 1.14	7.87 ± 1.17	0.04^{NS}	13,767 ± 3,927	14,058 ± 1,935	0.16^{NS}	31.17 ± 4.58	29.00 ± 2.53	1.01^{NS}

NS – Non-significant

* - Significant at ($p<0.05$)

Table 3b. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on the haematological parameters of crossbred calves (n=6)

Sl. No.	Sampling periods	Haemoglobin (g/dl)		Total erythrocyte count (millions/µl)		Total leucocyte count (numbers/µl)		Volume of packed red blood corpuscles (%)	
		Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
A	Before the start of the trial (180 d)	7.25 ± 0.57	7.17 ± 0.82	6.99 ± 1.46	7.09 ± 1.81	10,925 ± 3,914	10,708 ± 2,560	25.67 ± 3.39	24.50 ± 2.26
B	At the end of the trial (270 d)	8.21 ± 1.90	8.29 ± 0.62	7.84 ± 1.14	7.87 ± 1.17	13,767 ± 3,927	14,058 ± 1,935	31.17 ± 4.58	29.00 ± 2.53
C	One month after the end of the trial (300 d)	7.17 ± 1.17	7.17 ± 0.92	7.52 ± 1.98	7.19 ± 1.61	12,342 ± 2,823	12,700 ± 2,539	25.50 ± 3.39	26.17 ± 3.66
't' value for A vs B		1.25^{NS}	3.30^{*}	1.10^{NS}	1.13^{NS}	1.73^{NS}	2.29^{*}	3.51^{*}	2.87^{*}
't' value for A vs C		0.10^{NS}	0.11^{NS}	0.56^{NS}	0.18^{NS}	1.00^{NS}	1.27^{NS}	0.28^{NS}	0.70^{NS}
't' value for B vs C		1.67^{NS}	2.31^{*}	0.36^{NS}	0.80^{NS}	0.78^{NS}	1.79^{NS}	2.40^{*}	2.06^{*}

NS – Non-significant

* - Significant at ($p<0.05$)

Fig.2. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on haemoglobin content, total erythrocyte (RBC) count and volume of packed red blood corpuscles (VPRC) of crossbred calves

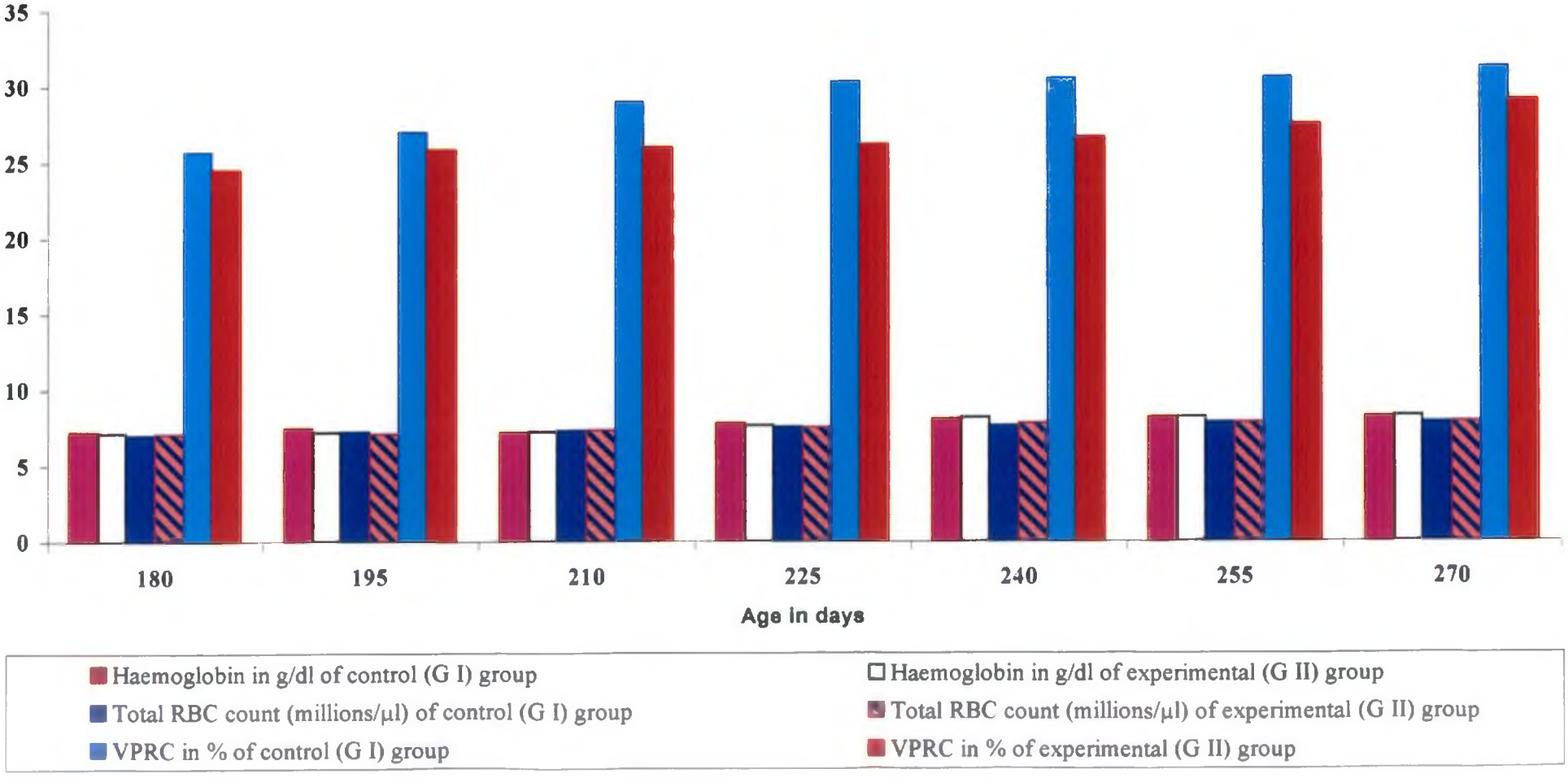
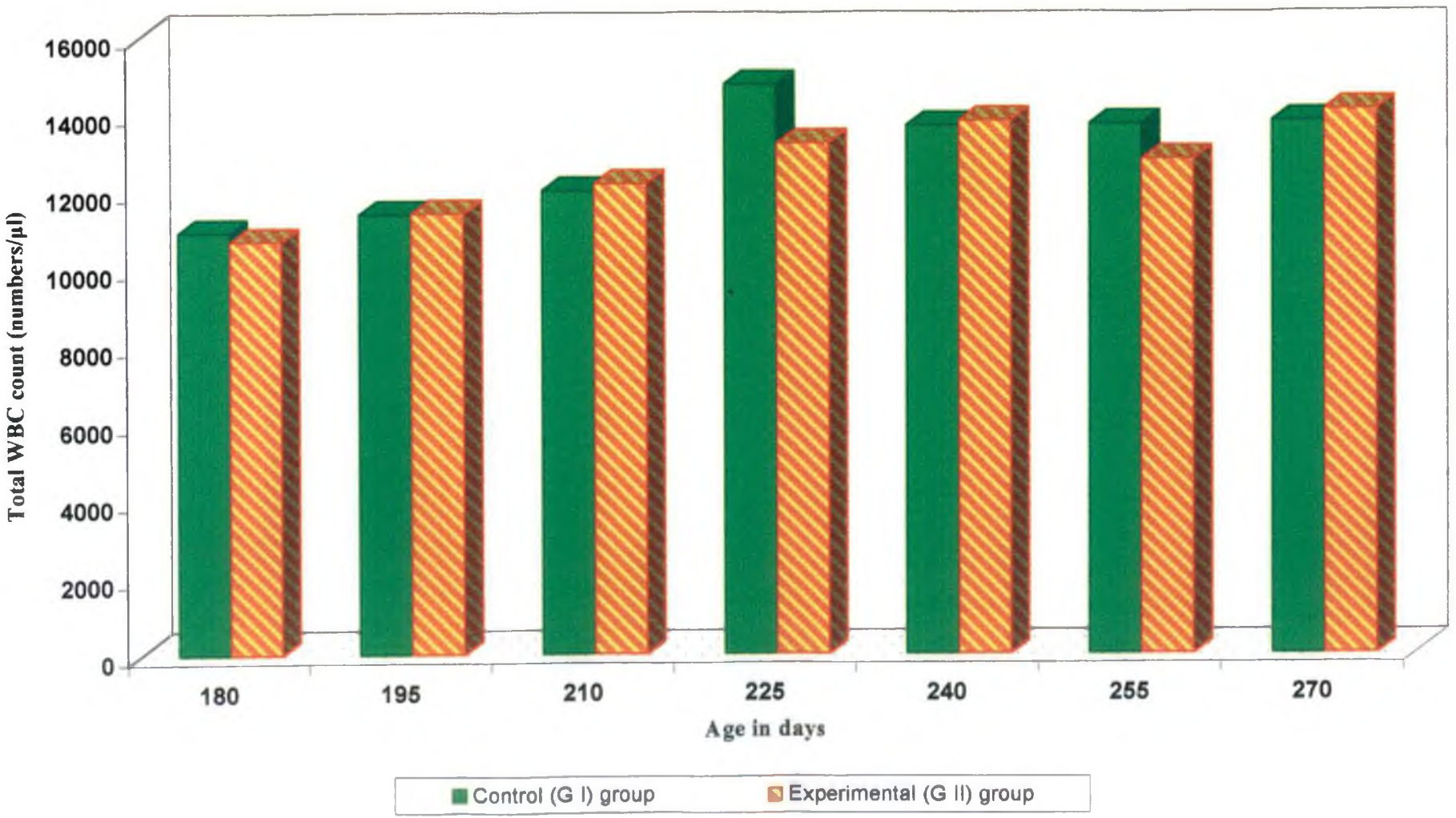


Fig.3. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on total leucocyte (WBC) count of crossbred calves



All comparisons between animals of G I and G II revealed statistically non-significant differences with particular reference to VPRC (table 3a).

4.3.5 Erythrocytic indices

The effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on erythrocytic indices viz., mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are presented in tables 3c, 3d and fig.4.

4.3.5.1 Mean corpuscular volume (MCV)

MCV showed a highly fluctuating trend in animals of both control (G I) and experimental (G II) groups during the trial period from 180 d to 270 d.

In animals of G I, MCV started at a value of 37.96 ± 8.86 fl at the start of the trial (180 d) vide table 3c and pursued a fluctuating tendency to reach the highest value of 41.09 ± 11.41 fl at the end of the trial (270 d). The MCV of calves of G I then declined sharply to reach the value of 35.29 ± 7.23 fl, one month after the end of the trial (table 3d).

The MCV in animals of G II pursued a similar fluctuating trend as that of animals of G I. But here, the MCV showed a starting value of 36.00 ± 7.34 fl on 180 d (table 3c) and followed fluctuating path to attain the lowest value of 35.24 ± 7.52 fl on 240 d and again increased to reach the highest value of 37.59 ± 6.40 fl on 270 d (table 3c). But unlike in animals of G I, the MCV of animals

of G II increased to attain the maximum value of 38.02 ± 10.60 fl, one month after the end of the trial (300 d) vide table 3d.

Statistically non-significant differences were revealed on comparisons between animals of G I and G II and within group with respect to MCV (tables 3c and 3d).

4.3.5.2 Mean corpuscular haemoglobin (MCH)

Little notable changes with meagre fluctuations were found in calves of both control (G I) and experimental (G II) groups with respect to MCH.

The MCH of animals of G I showed an initial value of 10.80 ± 2.56 pg at 180 d (table 3c), which increased to the highest value of 11.01 ± 3.88 pg within the next fortnight (195 d). Then the value showed a fluctuating as well as decreasing trend to reach the baseline normal value of 10.80 ± 3.49 pg on 270 d (at the end of the trial) vide table 3c, which further decreased to 9.96 ± 2.55 pg, one month after the end of the trial (300 d) vide table 3d.

Calves of G II showed an initial MCH value of 10.61 ± 2.88 pg on 180 d (table 3c) which followed a fluctuating path to reach the highest value of 10.91 ± 4.44 pg on 225 d and again pursued a decreasing tendency with little fluctuations to reach the final value of 10.67 ± 1.12 pg on 270 d (table 3c). As the observations of animals of G I, the MCH of animals of G II also decreased to a value of 10.31 ± 2.40 pg on 300 d (table 3d).

Statistical analysis employing student *t* test revealed non-significant differences while comparing between MCH values of animals of G I and G II (table 3c).

4.3.5.3 Mean corpuscular haemoglobin concentration (MCHC)

Like the other two erythrocytic indices, there was a fluctuating trend in the MCHC values of animals of control (G I) and experimental (G II) groups from 180 d to 270 d.

The MCHC of animals of G I showed a high initial value of 28.68 ± 4.69 g/dl at 180 d and then pursued a fluctuating and decreasing trend to reach the lowest value of 26.43 ± 4.99 g/dl at the end of the trial (270 d) vide table 3c. Then, the value increased to reach a higher value of 28.54 ± 6.03 g/dl at the end of the trial (300 d) vide table 3d.

Unlike animals of G I, a fluctuating tendency was observed in MCHC values of animals of G II. They showed an initial MCHC value of 29.48 ± 4.43 g/dl at 180 d of age (table 3c) which had a fluctuating trend to reach the highest value of 30.98 ± 4.62 g/dl at 240 d and then started declining to reach a value of 28.69 ± 2.27 g/dl at 270 d of age (at the end of the trial) vide table 3c. The value further declined to 27.56 ± 3.22 g/dl at 300 d (table 3d).

The fluctuating differences between MCHC values of animals of G I and G II were found to be statistically non-significant (table 3c) on employing student *t* test.

Table 3c. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on erythrocytic indices of crossbred calves (n=6)

Age in days	Mean corpuscular volume-MCV (fl)			Mean corpuscular haemoglobin-MCH (pg)			Mean corpuscular haemoglobin concentration-MCHC (g/dl)		
	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value
180	37.96 ± 8.86	36.00 ± 7.34	0.42 ^{NS}	10.80 ± 2.56	10.61 ± 2.88	0.12 ^{NS}	28.68 ± 4.69	29.48 ± 4.43	0.30 ^{NS}
195	39.04 ± 10.31	37.53 ± 7.33	0.29 ^{NS}	11.01 ± 3.88	10.50 ± 2.32	0.28 ^{NS}	28.21 ± 5.67	28.11 ± 3.69	0.04 ^{NS}
210	40.26 ± 4.07	36.59 ± 8.20	0.98 ^{NS}	10.37 ± 2.81	10.19 ± 2.47	0.12 ^{NS}	25.55 ± 5.26	28.18 ± 5.70	0.83 ^{NS}
225	38.28 ± 9.72	35.93 ± 7.12	0.48 ^{NS}	10.18 ± 3.62	10.91 ± 4.44	0.31 ^{NS}	26.76 ± 7.65	29.78 ± 8.09	0.67 ^{NS}
240	40.75 ± 8.49	35.24 ± 7.52	1.19 ^{NS}	10.80 ± 3.40	10.70 ± 1.56	0.07 ^{NS}	26.91 ± 7.56	30.98 ± 4.62	1.13 ^{NS}
255	39.68 ± 7.75	35.40 ± 3.96	1.20 ^{NS}	10.48 ± 2.69	10.51 ± 0.76	0.03 ^{NS}	27.14 ± 7.33	29.97 ± 3.65	0.85 ^{NS}
270	41.09 ± 11.41	37.59 ± 6.40	0.66 ^{NS}	10.80 ± 3.49	10.67 ± 1.12	0.09 ^{NS}	26.43 ± 4.99	28.69 ± 2.27	1.01 ^{NS}

NS – Non-significant

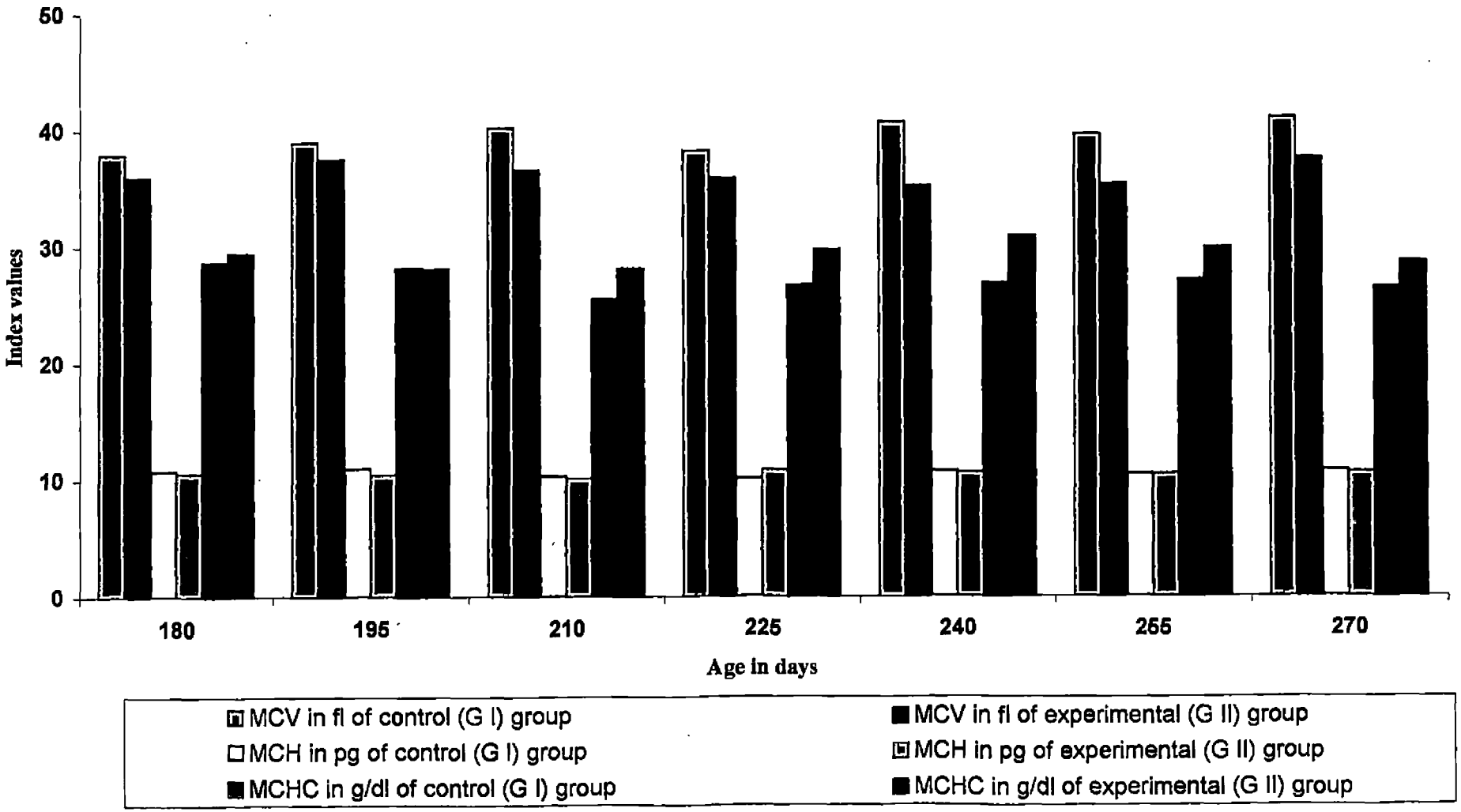
* - Significant at ($p<0.05$)

Table 3d. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on erythrocytic indices of crossbred calves (n=6)

Sl. No.	Sampling periods	Mean corpuscular volume- MCV (fl)		Mean corpuscular haemoglobin-MCH (pg)		Mean corpuscular haemoglobin concentration- MCHC (g/dl)	
		Group I	Group II	Group I	Group II	Group I	Group II
A	Before the start of the trial (180 d)	37.96 ± 8.86	36.00 ± 7.34	10.80 ± 2.56	10.61 ± 2.88	28.68 ± 4.69	29.48 ± 4.43
B	At the end of the trial (270 d)	41.09 ± 11.41	37.59 ± 6.40	10.80 ± 3.49	10.67 ± 1.12	26.43 ± 4.99	28.69 ± 2.27
C	One month after the end of the trial (300 d)	35.29 ± 7.23	38.02 ± 10.60	9.96 ± 2.55	10.31 ± 2.40	28.54 ± 6.03	27.56 ± 3.22
't' value for A vs B		0.90^{NS}	0.16^{NS}	0.06^{NS}	0.03^{NS}	1.15^{NS}	0.20^{NS}
't' value for A vs C		0.44^{NS}	0.24^{NS}	0.58^{NS}	0.31^{NS}	0.22^{NS}	0.75^{NS}
't' value for B vs C		1.05^{NS}	0.09^{NS}	0.48^{NS}	0.33^{NS}	0.66^{NS}	0.70^{NS}

NS – Non-significant
* - Significant at ($p<0.05$)

Fig.4. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on erythrocytic indices viz., MCV, MCH and MCHC of crossbred calves



4.4 Biochemical parameters

4.4.1 Serum protein profile

The effects of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum protein profile of crossbred calves are given in tables 4a, 4b and fig.5.

4.4.1.1 Total protein

There found an upstream increasing trend in serum total protein content of animals of both control (G I) and experimental (G II) groups during the entire study period of 180 d to 270 d of age.

The serum total protein content of animals of G I pursued an increasing trend (table 4a) and attained the highest value of 7.40 ± 0.39 g/dl at the end of trial (270 d), which was found to be significantly ($P<0.05$) higher when compared to the value (6.52 ± 0.38 g/dl) at the start of the experiment (180 d) vide table 4b. A slight decline of total protein content of animals of G I was then noticed to reach the value of 7.23 ± 0.60 g/dl which was also significantly ($P<0.05$) higher compared to the 180 d value of 6.52 ± 0.38 g/dl (table 4b).

As in calves of G I, the total protein content of animals of G II pursued a steeply increasing trend (table 4a) to attain the highest value of 7.70 ± 1.00 g/dl on 270 d, which was significantly ($P<0.05$) higher than the starting 180 d value of 6.51 ± 0.21 g/dl (table 4b). As the trend in animals of G I, the total protein of calves of G II declined sharply to reach the value of 7.21 ± 0.33 g/dl which

was significantly ($P<0.05$) higher compared to the 180 d value of 6.51 ± 0.21 g/dl (table 4b).

All comparisons between animals of G I and G II, employing student *t* test revealed non-significant differences in total protein content (table 4a).

4.4.1.2 Albumin

The concentration of serum albumin of calves of both control (G I) and experimental (G II) groups showed a gradual increasing trend as that of total protein from 180 d to 270 d of age.

The serum albumin concentration of calves of G I attained the highest value of 3.59 ± 0.37 g/dl at the end of the trial (270 d) which was significantly ($P<0.05$) higher than the initial level of 2.99 ± 0.41 g/dl (180 d) vide tables 4a and4b. Serum albumin concentration then decreased on 3.32 ± 0.09 g/dl, one month after the end of the trial (300 d) vide table 4b.

Animals of G II showed the similar trend as that of animals of G I (table 4a) and attained the higher values from 255 d onwards to reach the highest value of 3.63 ± 0.50 g/dl on 270 d which was significantly ($P<0.05$) higher when compared to the 180 d value of 3.18 ± 0.33 g/dl (table 4a). As in animals of G I, the albumin concentration declined to 3.18 ± 0.43 on 300 d (table 4b).

Statistical analysis using student *t* test between animals of G I and G II revealed non-significant differences with respect to serum albumin concentration (table 4a).

4.4.1.3 Globulin

Animals of both control (G I) and experimental (G II) groups had significant differences in serum globulin content during the experimental period (180 d to 270 d) vide table 4a.

Calves of G I showed a globulin content of 3.53 ± 0.55 g/dl at the initial day of the trial (180 d) and pursued a decreasing trend till 210 d and then followed an increasing trend to reach the highest value of 3.81 ± 0.69 g/dl at the end of the trial of 270 d (table 4a). There was an increasing trend to reach the value of 3.90 ± 0.57 g/dl, one month after the end of the trial (300 d) vide table 4b.

Unlike animals of G I, calves of G II showed a continuously increasing trend in serum globulin content (table 4a) which reached the peak value of 4.07 ± 0.72 g/dl on 270 d, which was significantly ($P<0.05$) higher when compared to the 180 d value of 3.33 ± 0.20 g/dl (table 4b). The globulin content then slightly declined to attain a value of 4.02 ± 0.40 g/dl on 300 d, which was also significantly ($P<0.05$) higher than that of the value at 180 d (3.33 ± 0.20 g/dl) vide table 4b.

As that of other protein parameters, serum globulin content revealed a non-significant differences between the animals of G I and G II (table 4a).

4.4.1.4. Albumin: Globulin (A:G) ratio

Fluctuating tendency was evident in the A:G ratio of calves of both control (G I) and experimental (G II) groups during the trial period from 180 d to 270 d of age.

A:G ratio of animals of G I exhibited a fluctuating trend (table 4a), with the value of 0.87 ± 0.23 on 180 d and reached the highest value of 1.06 ± 0.38 at 225 d and then lowered to attain the value of 0.98 ± 0.23 at the age of 270 d (table 4a). It further decreased to the value of 0.87 ± 0.12 one month after the end of the trial (300 d) vide table 4b.

As in the case of animals of G I, A:G ratio of animals of G II pursued a fluctuating trend (table 4a) with a value of 0.96 ± 0.15 on 180 d and reached the highest value of 1.01 ± 0.41 on 225 d which again reduced to a baseline value of 0.91 ± 0.20 on 270 d (table 4b). A:G ratio of calves of experimental group, like that of animals of control group, further declined to the value of 0.80 ± 0.17 on 300 d (table 4b).

Non-significant difference was evident between animals of both G I and G II and within group as far as A:G ratio was concerned (table 4a).

Table 4a. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum protein profile of crossbred calves (n=6)

Age in days	Total Protein (g/dl)			Albumin (g/dl)			Globulin (g/dl)			A:G ratio		
	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value
180	6.52 ± 0.38	6.51 ± 0.21	0.01 ^{NS}	2.99 ± 0.41	3.18 ± 0.33	0.89 ^{NS}	3.53 ± 0.55	3.33 ± 0.20	0.81 ^{NS}	0.87 ± 0.23	0.96 ± 0.15	0.80 ^{NS}
195	6.65 ± 0.18	6.83 ± 0.94	0.47 ^{NS}	3.21 ± 0.47	3.20 ± 0.48	0.02 ^{NS}	3.44 ± 0.54	3.47 ± 0.96	0.05 ^{NS}	0.97 ± 0.28	1.00 ± 0.34	0.18 ^{NS}
210	6.69 ± 0.43	6.94 ± 0.61	0.83 ^{NS}	3.32 ± 0.73	3.29 ± 0.72	0.08 ^{NS}	3.37 ± 0.72	3.55 ± 0.46	0.82 ^{NS}	1.05 ± 0.39	0.92 ± 0.28	0.67 ^{NS}
225	6.75 ± 0.40	7.07 ± 0.77	0.89 ^{NS}	3.37 ± 0.38	3.37 ± 0.60	0.03 ^{NS}	3.38 ± 0.68	3.69 ± 1.17	0.57 ^{NS}	1.06 ± 0.38	1.01 ± 0.41	0.22 ^{NS}
240	7.21 ± 0.56	7.25 ± 0.74	0.10 ^{NS}	3.48 ± 0.30	3.47 ± 0.50	0.03 ^{NS}	3.73 ± 0.73	3.78 ± 0.99	0.09 ^{NS}	0.98 ± 0.30	1.00 ± 0.41	0.12 ^{NS}
255	7.27 ± 0.71	7.42 ± 0.29	0.50 ^{NS}	3.56 ± 0.31	3.63 ± 0.38	0.33 ^{NS}	3.71 ± 0.80	3.80 ± 0.36	0.26 ^{NS}	1.00 ± 0.25	0.97 ± 0.18	0.28 ^{NS}
270	7.40 ± 0.39	7.70 ± 1.00	0.68 ^{NS}	3.59 ± 0.37	3.63 ± 0.50	0.13 ^{NS}	3.81 ± 0.69	4.07 ± 0.72	0.64 ^{NS}	0.98 ± 0.23	0.91 ± 0.20	0.53 ^{NS}

NS – Non-significant

* - Significant at ($p<0.05$)

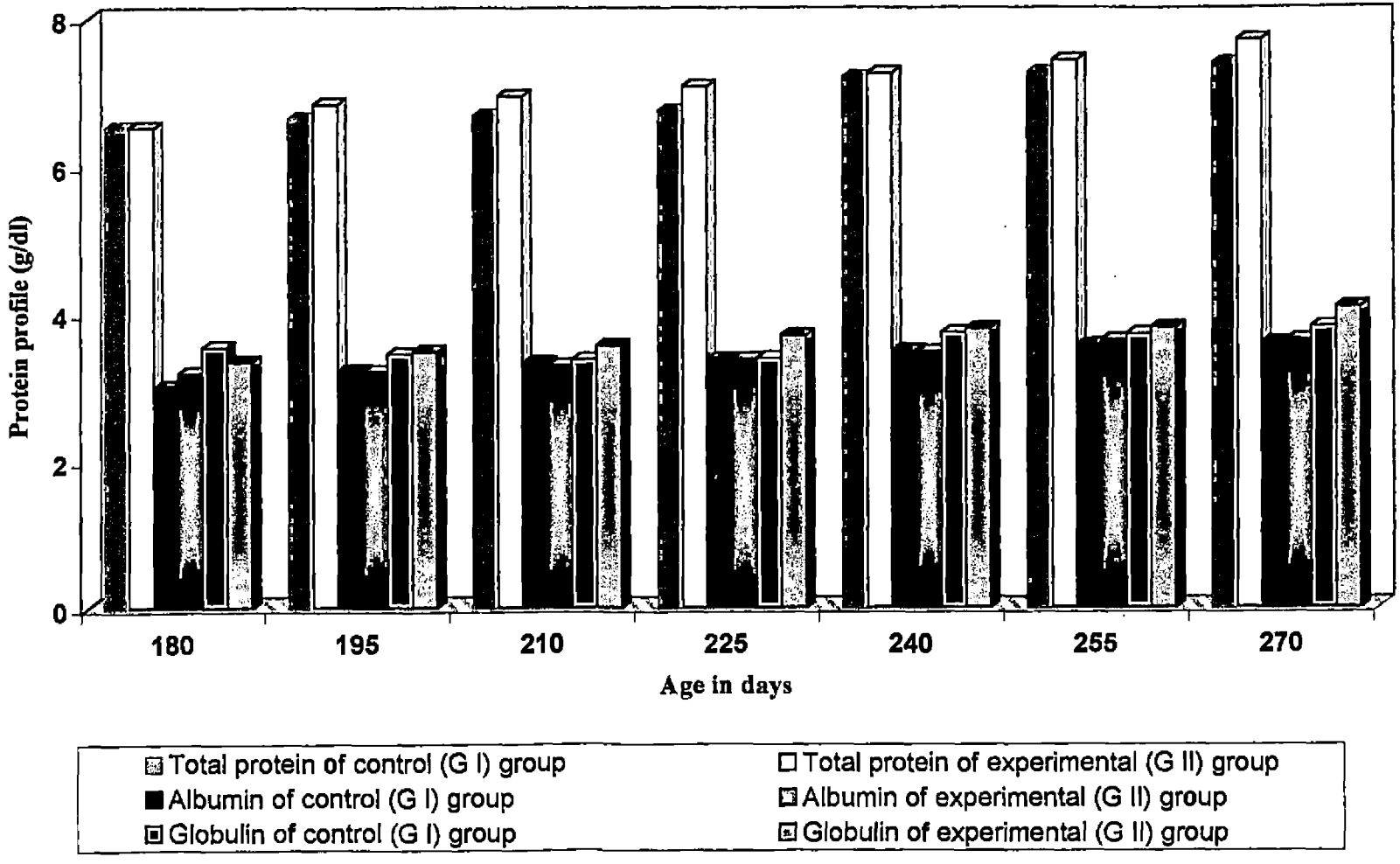
Table 4b. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum protein profile of crossbred calves (n=6)

Sl. No.	Sampling periods	Total Protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		A:G ratio	
		Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
A	Before the start of the trial (180 d)	6.52 ± 0.38	6.51 ± 0.21	2.99 ± 0.41	3.18 ± 0.33	3.53 ± 0.55	3.33 ± 0.20	0.87 ± 0.23	0.96 ± 0.15
B	At the end of the trial (270 d)	7.40 ± 0.39	7.70 ± 1.00	3.59 ± 0.37	3.63 ± 0.50	3.81 ± 0.69	4.07 ± 0.72	0.98 ± 0.23	0.91 ± 0.20
C	One month after the end of the trial (300 d)	7.23 ± 0.60	7.21 ± 0.33	3.32 ± 0.09	3.18 ± 0.43	3.90 ± 0.57	4.02 ± 0.40	0.87 ± 0.12	0.80 ± 0.17
't' value for A vs B		5.43*	2.84*	2.72*	2.59*	1.48 NS	2.44*	0.58 NS	0.07 NS
't' value for A vs C		2.76*	4.54*	2.08*	0.50 NS	2.03 NS	2.93*	0.60 NS	1.24 NS
't' value for B vs C		0.65 NS	1.36 NS	1.73 NS	1.60 NS	0.23 NS	0.12 NS	0.90 NS	0.77 NS

NS – Non-significant

* - Significant at ($p<0.05$)

Fig.5. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum protein profile of crossbred calves



4.4.2. Serum lipid profile.

The effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum lipid profile of crossbred calves are presented in tables 5a, 5b and fig. 6 and 7.

4.4.2.1. Total lipids

There was an increasing trend in the concentration of total lipids of calves of both control (G I) and experimental (G II) groups during the experimental period from 180 d to 270 d of age.

Animals of G I showed a clear cut upstream trend in the concentration of total lipids (table 5a) which reached the highest value of 276.16 ± 59.45 mg/dl at the end of the trial (270 d) when compared to the value of 229.90 ± 95.41 mg/dl at the initial stage of the experiment (180 d) vide table 5a. The concentration reached to the baseline value of 225.18 ± 27.92 mg/dl one month after the end of the trial (300 d) vide table 5b.

The total lipids concentration of animals of G II also pursued an increasing trend as animals of G I, reaching the peak value of 278.26 ± 42.78 mg/dl on 270 d, in comparison to the value of 242.77 ± 73.05 mg/dl at the initial stage of the trial (180 d) vide table 5a. As in calves of G I, the total lipids declined to a value of 234.20 ± 69.47 mg/dl at 300 d (table 5b).

All comparisons between animals of both G I and G II were found to be statistically non-significant with respect to the concentration of serum total lipids (table 5a).

4.4.2.2. Cholesterol

Increasing trend in the concentration of cholesterol of animals of control (G I) and experimental (G II) groups was evident during the trial period from 180 d to 270 d of age.

Animals of G I exhibited an upstream trend in the concentration of cholesterol (table 5a) with a value of 51.37 ± 19.21 mg/dl at the start of the trial (180 d) which increased to attain the highest value of 61.63 ± 16.57 mg/dl on 255 d and reached a value of 56.30 ± 14.96 mg/dl at the end of the trial (270 d) vide table 5a. The cholesterol content was then slightly elevated to a value of 56.51 ± 5.14 mg/dl, one month after the end of the trial (300 d) vide table 5b.

Unlike animals of G I, the concentration of cholesterol of animals of G II showed a fluctuating trend during the trial period with the highest value of 59.82 ± 22.70 mg/dl on 240 d, and attained a value of 57.52 ± 16.80 mg/dl on 270 d, when compared to the 180 d value of 52.46 ± 16.21 mg/dl (table 5a). The cholesterol content, finally, lowered to a value of 55.04 ± 12.87 mg/dl on 300 d (table 5b).

Statistical analysis using unpaired student *t* test revealed non-significant differences in the serum concentration of cholesterol between animals of both G I and G II (table 5a).

4.4.2.3. Triglycerides

There found a definite increasing trend in the concentration of triglycerides of calves of both control (G I) and experimental (G II) groups during the experimental period (180 d to 370 d).

Upstream tendency was quite evident in the concentration of triglycerides of animals of G I (table 5a) which reached the highest value of 23.21 ± 9.44 mg/dl at the end of the trial (270 d) in comparison to a triglyceride concentration of 20.31 ± 3.19 mg/dl at the initial stage of experiment (180 d) vide table 5a. The concentration later declined to 19.57 ± 1.03 mg/dl one month after the end of the trial (300 d) vide table 5b.

The upstream tendency noticed in animals of G I was repeated in the concentration of triglycerides of animals of G II (table 5a) which was elevated to the highest value of 23.80 ± 4.17 mg/dl on 270 d in comparison to the 180 d value of 19.99 ± 7.35 mg/dl (table 5a). The concentration of triglycerides finally settled at the baseline normal value of 20.20 ± 0.95 mg/dl on 300 d, as in animals of G I.

Significantly no differences could be observed in the concentration of triglycerides between animals of G I and G II, while student *t* test comparison was employed (table 5a).

4.4.2.4. Non-esterified fatty acids (NEFA)

Fluctuating yet decreasing trend was evident in the concentration of NEFA of crossbred calves of both control (G I) and experimental (G II) groups during the trial period from 180 d to 270 d of age.

Concentration of NEFA of animals of G I revealed a fluctuating trend (table 5a) and reached a value of 387.99 ± 67.73 μmol/l at the end of the trial (270 d) which was significantly ($P<0.05$) lower than the value 453.08 ± 77.21 μmol/l obtained at the start of the trial (180 d) vide table 5b. The NEFA concentration was again elevated to a value of 477.76 ± 91.52 μmol/l one month after the end of the trial (300 d) vide table 5b.

The fluctuating trend noticed in animals of G I was also reflected in the concentration of NEFA of animals of G II which reached the lowest value of 400.16 ± 64.76 μmol/l on 270 d in comparison to a concentration of 456.59 ± 26.06 μmol/l at the initial stage of the trial (180 d) vide table 5a. The NEFA concentration was again elevated to a value of 461.68 ± 64.43 μmol/l on 300 d (table 5b).

All comparisons between animals of both G I and G II employing student *t* test revealed significant ($P<0.05$) differences in the concentration of NEFA on 225 d with the corresponding values of 374.27 ± 53.24 μmol/l and 449.12 ± 57.47 μmol/l for animals of G I and G II respectively (table 5a).

Table 5a. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum lipid profile of crossbred calves (n=6)

Age in days	Total lipids (mg/dl)			Cholesterol (mg/dl)			Triglycerides (mg/dl)			Non-esterified fatty acids (µmol/l)		
	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value
180	229.90 ± 95.41	242.77 ± 73.05	0.26^{NS}	51.37 ± 19.21	52.46 ± 16.21	0.11^{NS}	20.31 ± 3.19	19.99 ± 7.35	0.10^{NS}	453.08 ± 77.21	456.59 ± 26.06	0.11^{NS}
195	233.95 ± 13.48	261.41 ± 29.90	2.05^{NS}	52.86 ± 9.64	49.80 ± 13.03	0.46^{NS}	20.56 ± 6.37	20.40 ± 6.49	0.04^{NS}	463.55± 81.65	490.38 ± 43.06	0.71^{NS}
210	253.00 ± 54.86	262.51 ± 42.25	0.34^{NS}	57.11 ± 14.01	51.09 ± 13.78	0.75^{NS}	20.92 ± 5.08	21.40 ± 3.51	0.19^{NS}	413.89± 86.12	432.98 ± 81.96	0.39^{NS}
225	262.02 ± 38.81	276.17 ± 46.00	0.58^{NS}	57.23 ± 10.78	52.61 ± 14.13	0.64^{NS}	21.71 ± 7.29	21.34 ± 6.73	0.09^{NS}	374.27 ± 53.24	449.12± 57.47	2.34*
240	263.24 ± 57.15	276.36 ± 49.67	0.42^{NS}	55.68 ± 13.31	59.82 ± 22.70	0.38^{NS}	22.50 ± 8.23	22.34 ± 3.33	0.04^{NS}	363.80 ± 27.70	413.00 ± 52.31	2.04^{NS}
255	275.15 ± 50.49	276.44 ± 50.14	0.04^{NS}	61.63 ± 16.57	58.09 ± 22.49	0.31^{NS}	22.63 ± 5.67	23.78 ± 6.49	0.33^{NS}	405.28 ± 64.58	423.19 ± 98.32	0.37^{NS}
270	276.16 ± 59.45	278.26 ± 42.78	0.07^{NS}	56.30 ± 14.96	57.52 ± 16.80	0.13^{NS}	23.21 ± 9.44	23.80 ± 4.17	0.14^{NS}	387.99 ± 67.73	400.16 ± 64.76	0.32^{NS}

NS – Non-significant

* - Significant at ($p<0.05$)

Table 5b. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum lipid profile of crossbred calves (n=6)

Sl. No.	Sampling periods	Total lipids (mg/dl)		Cholesterol (mg/dl)		Triglycerides (mg/dl)		Non-esterified fatty acids (µmol/l)	
		Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
A	Before the start of the trial (180 d)	229.90 ± 95.41	242.77 ± 73.05	51.37 ± 19.21	52.46 ± 16.21	20.31 ± 3.19	19.99 ± 7.35	453.08 ± 77.21	456.59 ± 26.06
B	At the end of the trial (270 d)	276.16 ± 59.45	278.26 ± 42.78	56.30 ± 14.96	57.52 ± 16.80	23.21 ± 9.44	23.80 ± 4.17	387.99 ± 67.73	400.16 ± 64.76
C	One month after the end of the trial (300 d)	225.18 ± 27.92	234.20 ± 69.47	56.51 ± 5.14	55.04 ± 12.87	19.57 ± 1.03	20.20 ± 0.95	477.76 ± 91.52	461.68 ± 64.43
't' value for A vs B		1.06 NS	1.17 NS	0.51 NS	0.64 NS	0.88 NS	1.45 NS	2.26 *	1.88 NS
't' value for A vs C		0.43 NS	0.05 NS	0.83 NS	0.37 NS	0.36 NS	0.03 NS	0.67 NS	0.24 NS
't' value for B vs C		2.01 NS	1.79 NS	0.03 NS	0.27 NS	0.89 NS	2.34 NS	2.02 NS	2.44 NS

NS – Non-significant
* - Significant at ($p<0.05$)

Fig.6. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum lipid profile of crossbred calves

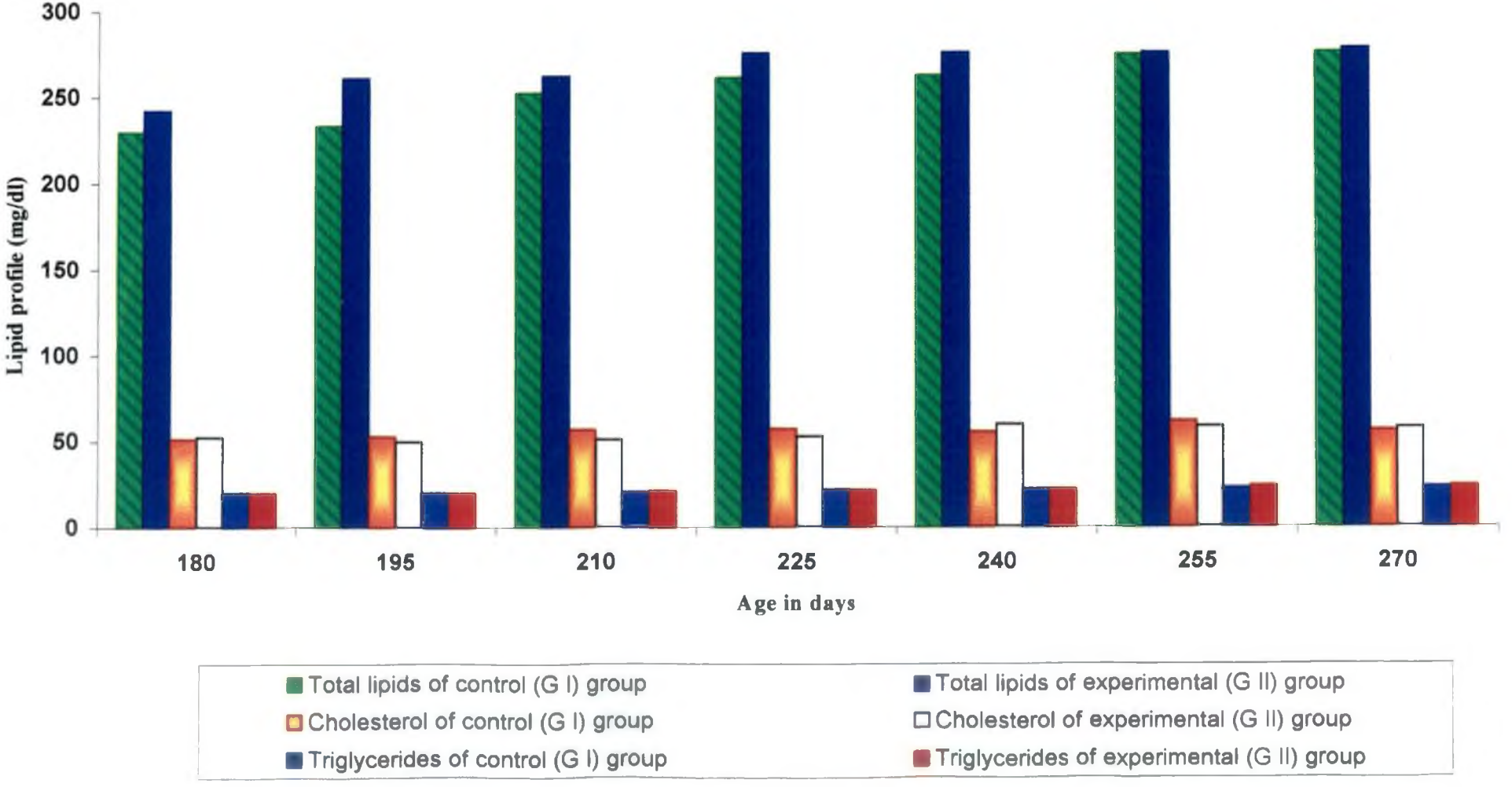
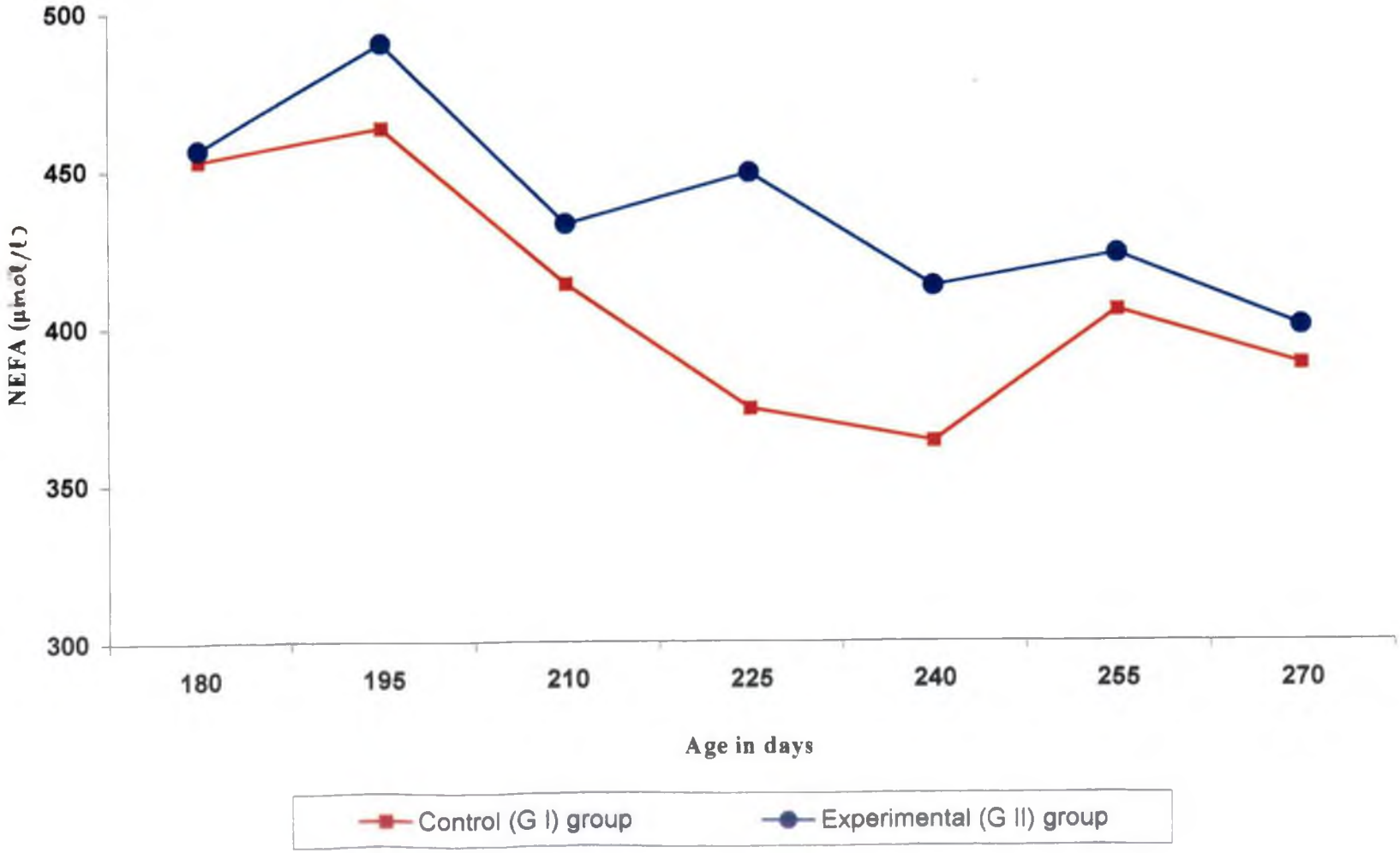


Fig.7. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on non-esterified fatty acids (NEFA) of crossbred calves



4.4.3. Blood glucose level (BGL)

The effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on blood glucose level (BGL) of crossbred calves is presented in tables 6a and 6b and Figure 8. There was an increasing trend in BGL of animals of control (G I) and experimental (G II) groups during the experimental period of 180 d to 270 days of age.

An upstream tendency in BGL of animals of G I was quite evident which reached the peak value of 76.00 ± 20.66 mg/dl at the end of the trial (270 d) vide table 6a when compared to the lowest value of 69.00 ± 7.04 mg/dl at the initial period of the experiment (180 d) vide table 5a. The BGL then declined to the baseline normal value of 66.83 ± 6.24 mg/dl one month after the end of the trial (300 d) vide table 6b.

The increasing tendency of BGL found in animals of G I was also noticed in the BGL of crossbred calves of G II (table 6a) which reached the highest value of 78.50 ± 10.73 mg/dl at 270 d of age, that was significantly ($P<0.05$) higher when compared to the starting 180 d BGL value of 64.33 ± 6.80 mg/dl (table 6b). The BGL of animals of G II then lowered to a value of 64.83 ± 10.74 mg/dl at 300 d (table 6b).

There was no significant variation in BGL on comparison between animals of G I and G II, employing student *t* test (table 6a).

4.4.4 Serum urea nitrogen (SUN)

The effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum urea nitrogen of crossbred calves is given in tables 6a, 6b and fig.9. Serum urea nitrogen (SUN) showed a continuous increasing trend during the experimental period from 180 d to 270 d in crossbred calves of both control (G I) and experimental (G II) groups.

The upstream trend was quite evident in SUN of animals of G I (table 6a) which reached the highest value of 19.55 ± 2.98 mg/dl at the end of the trial (270 d), which was significantly ($P<0.05$) higher when compared to the value of 14.96 ± 2.62 mg/dl at the initial stage of the experimental period (180 d) vide table 6b. Then, the urea nitrogen content declined to the baseline normal value of 14.53 ± 2.18 mg/dl, one month after the end of the trial which was significantly ($P<0.05$) lower when compared with 270 d value of 19.55 ± 2.98 mg/dl (table 6b).

The rising trend noticed in animals of G I was also reflected in the BUN content of animals of G II (table 6a), which was elevated to the maximum value of 20.71 ± 3.44 mg/dl on 270 d, which was significantly ($P<0.05$) higher than the 180 d value of 13.66 ± 1.65 mg/dl (table 6b) and then, as in animals of G I, the urea nitrogen of animals of G II reduced to a significantly ($P<0.05$) lower value of 13.49 ± 1.78 mg/dl on 300 d when compared to the 270 d value (table 6b).

Serum urea content exhibited statistically no significant differences between animals of G I and G II (table 6a).

4.4.5 Creatinine

The effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum creatinine content of crossbred calves is shown in tables 6a and 6b and fig.9. A definite decreasing trend in the creatinine content of animals of both control (G I) and experimental (G II) groups was evident during the experimental period from 180 d to 270 d of age.

Animals of G I showed a decreasing trend in creatinine content (table 6a) and attained the lowest creatinine content of 0.73 ± 0.20 mg/dl at the end of the trial (255 d) when compared to the value of 0.83 ± 0.22 mg/dl at the initial period of the experiment (180 d) vide table 6a and the value was then increased to 0.79 ± 0.39 mg/dl one month after the end of the experiment (300 d) vide table 6b.

The downstream tendency shown by animals of G I was clearly reflected in the creatinine content of animals of G II, which started at the value of 0.88 ± 0.34 mg/dl on 180 d and reduced to the lowest value of 0.73 ± 0.34 mg/dl on 270 d (table 6a). The serum creatinine content, was elevated then to the baseline value of 0.87 ± 0.25 mg/dl on 300 d (table 6b).

Statistical analysis employing student *t* test exhibited non-significant differences in creatinine content between animals of G I and G II and within group (table 6a).

4.4.6 Bilirubin

The effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum bilirubin concentration of crossbred calves is presented in tables 6a, 6b. and fig.9. A decreasing trend was evident in the serum bilirubin concentration of crossbred calves of both control (G I) and experimental (G II) groups during the experimental period from 180 d to 225 d.

Bilirubin concentration of calves of G I pursued a decreasing trend, starting at a value of 0.70 ± 0.12 mg/dl at the start of the experiment (180 d) and reduced to the lower value of 0.59 ± 0.06 mg/dl at the end of the experiment (270 d) vide table 6a. One month after the end of the trial (300 d), the bilirubin concentration slightly increased to 0.62 ± 0.07 mg/dl (table 6b).

The downstream tendency in the bilirubin concentration was also evident in animals of G II, which showed the lowest value of 0.54 ± 0.17 mg/dl on 270 d when compared to the 180 d value of 0.59 ± 0.25 mg/dl (table 6a). Similar to the trend in animals of G I, the bilirubin concentration, increased to a value of 0.62 ± 0.09 mg/dl on 300 d (table 6b).

Table 6a. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on blood glucose level (BGL) and on serum biochemical parameters of crossbred calves (n=6)

Age in Days	Blood glucose level-BGL (mg/dl)			Urea Nitrogen (mg/dl)			Creatinine (mg/dl)			Bilirubin (mg/dl)		
	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value
180	69.00 ± 7.04	64.33 ± 6.80	1.17^{NS}	14.96 ± 2.62	13.66 ± 1.65	1.03^{NS}	0.83 ± 0.22	0.88 ± 0.34	0.29^{NS}	0.70 ± 0.12	0.59 ± 0.25	0.98^{NS}
195	69.83 ± 16.95	69.50 ± 14.12	0.04^{NS}	16.11 ± 2.16	16.46 ± 2.72	0.24^{NS}	0.81 ± 0.26	0.84 ± 0.22	0.27^{NS}	0.64 ± 0.15	0.65 ± 0.05	0.21^{NS}
210	72.33 ± 8.50	69.83 ± 14.15	0.37^{NS}	18.89 ± 2.78	17.05 ± 2.49	1.21^{NS}	0.80 ± 0.47	0.84 ± 0.22	0.20^{NS}	0.62 ± 0.19	0.63 ± 0.15	0.08^{NS}
225	72.33 ± 13.20	70.00 ± 9.40	0.35^{NS}	18.23 ± 3.13	18.22 ± 3.36	0.00^{NS}	0.78 ± 0.28	0.79 ± 0.31	0.03^{NS}	0.51 ± 0.18	0.55 ± 0.19	0.39^{NS}
240	75.00 ± 6.69	72.33 ± 32.47	0.54^{NS}	18.81 ± 2.87	18.41 ± 3.27	0.23^{NS}	0.76 ± 0.22	0.76 ± 0.22	0.01^{NS}	0.54 ± 0.25	0.54 ± 0.13	0.05^{NS}
255	75.67 ± 10.80	76.17 ± 11.41	0.08^{NS}	19.01 ± 4.29	18.92 ± 2.50	0.04^{NS}	0.73 ± 0.20	0.75 ± 0.30	0.14^{NS}	0.56 ± 0.19	0.57 ± 0.13	0.09^{NS}
270	76.00 ± 20.66	78.50 ± 10.73	0.26^{NS}	19.55 ± 2.98	20.71 ± 3.44	0.63^{NS}	0.76 ± 0.21	0.73 ± 0.34	0.15^{NS}	0.59 ± 0.06	0.54 ± 0.17	0.67^{NS}

NS – Non-significant

* - Significant at ($p<0.05$)

Table 6b. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on blood glucose level (BGL) and on serum biochemical parameters of crossbred calves (n=6)

Sl. No.	Sampling periods	Blood glucose level-BGL (g/dl)		Urea nitrogen (mg/dl)		Creatinine (mg/dl)		Bilirubin (mg/dl)	
		Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
A	Before the start of the trial (180 d)	69.00 ± 7.04	64.33 ± 6.80	14.96 ± 2.62	13.66 ± 1.65	0.83 ± 0.22	0.88 ± 0.34	0.70 ± 0.12	0.59 ± 0.25
B	At the end of the trial (270 d)	76.00 ± 20.66	78.50 ± 10.73	19.55 ± 2.98	20.71 ± 3.44	0.76 ± 0.20	0.73 ± 0.34	0.59 ± 0.06	0.54 ± 0.17
C	One month after the end of the trial (300 d)	66.83 ± 6.24	64.83 ± 10.74	14.53 ± 2.18	13.49 ± 1.78	0.79 ± 0.39	0.87 ± 0.25	0.62 ± 0.07	0.62 ± 0.09
't' value for A vs B		1.08^{NS}	2.83^{*}	4.26^{*}	4.84^{*}	0.78^{NS}	0.84^{NS}	0.89^{NS}	1.12^{NS}
't' value for A vs C		0.05^{NS}	0.44^{NS}	0.20^{NS}	0.79^{NS}	0.42^{NS}	0.09^{NS}	0.37^{NS}	0.21^{NS}
't' value for B vs C		1.29^{NS}	2.14^{*}	5.88^{*}	8.69^{*}	0.20^{NS}	0.91^{NS}	1.19^{NS}	1.18^{NS}

NS – Non-significant

* - Significant at ($p<0.05$)

Fig.8. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on blood glucose level (BGL) and on serum insulin concentration of crossbred calves

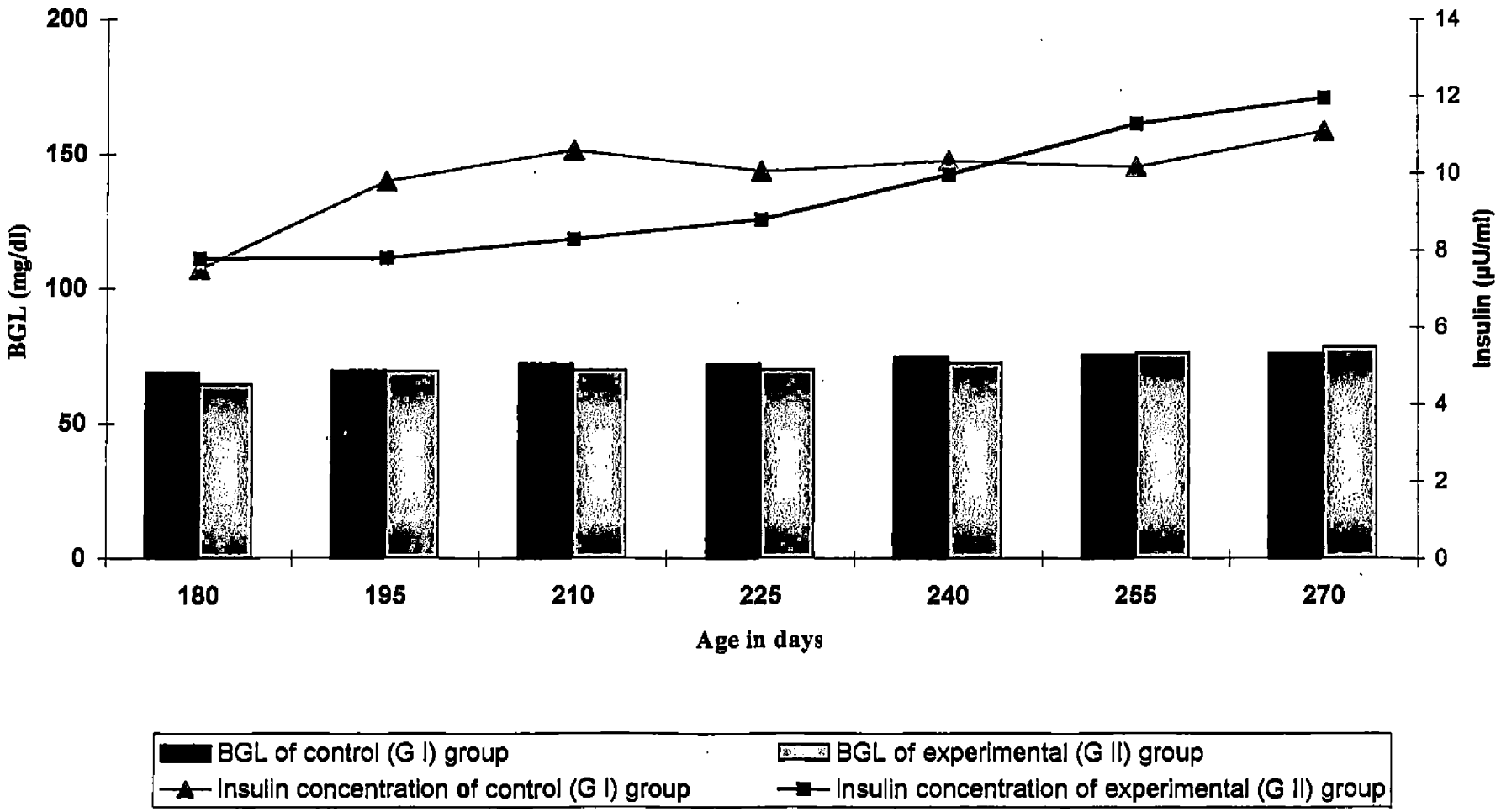
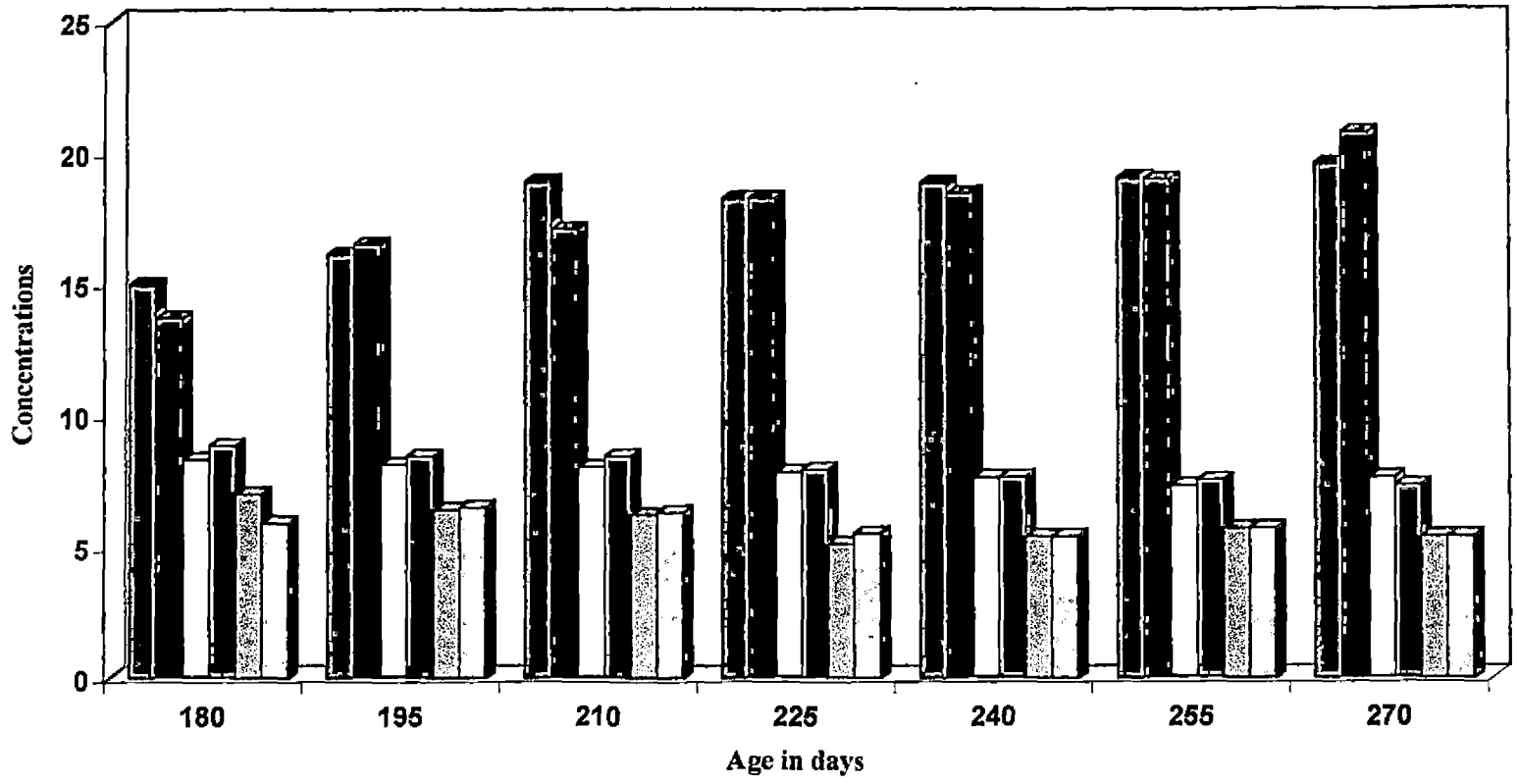


Fig.9. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum urea nitrogen, creatinine and bilirubin contents of crossbred calves



Bilirubin concentration did not show any statistically significant difference on comparison between animals of G I and G II and within group during the experimental period (tables 6a and 6b).

4.5 Serum hormonal traits

The effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on certain hormonal traits like thyroxine (T_4), triiodothyroxine (T_3) and insulin as well as thyroxine: triiodothyronine (T_4:T_3) ratio of crossbred calves during the study period from 180 d to 270 d of age are presented in tables 7a, 7b and fig. 8 and 10.

4.5.1 Thyroxine (T_4)

A fluctuating yet an increasing trend was clearly evident in T_4 concentration of crossbred calves of both control (G I) and experimental (G II) groups during the experimental period.

The serum T_4 concentration of animals of G I showed a fluctuating trend with an initial value of 37.38 ± 3.91 ng/ml at the age of 180 d and increased to the highest value of 42.98 ± 3.10 ng/ml on 255 d and reached 41.44 ± 5.16 ng/ml at the end of the experiment (270 d) vide table 7a. Then, the value reached the baseline normal level of 36.29 ± 8.18 ng/ml one month after the end of the experiment (300 d) vide table 7b.

Those fluctuations observed in animals of G I was also seen in serum T_4 concentration of animals of G II (table 7a). The serum T_4 concentration reached the highest value of 43.69 ± 3.53 ng/ml on 255 d and settled at a value

of 43.38 ± 3.53 ng/ml on 270 d, which was significantly (P<0.05) higher when compared to 180 d value of 37.06 ± 7.96 ng/ml. Similar to the trend in animals of G I, the T_4 concentration of animals of G II decreased to a value of 36.39 ± 3.94 ng/ml on 300 d, which was significantly (P<0.05) lower when compared to the 270 d value of 43.38 ± 3.53.

Serum T_4 concentration exhibited statistically non-significant differences while comparing between animals of G I and G II during the experimental period, on employing student *t* test (table 7a).

4.5.2 Triiodothyronine (T_3)

A fluctuating upstream trend was observed in the triiodothyronine (T_3) concentration of crossbred calves of both control (G I) and experimental (G II) groups during the experimental period from 180 d to 270 d.

Serum T_3 concentration of animals of G I revealed an increasing yet fluctuating trend (table 7a) and attained the highest value of 1.46 ± 0.25 ng/ml at the end of the trial (270 d) which was significantly (P<0.05) higher when compared to the value of 1.04 ± 0.11 ng/ml at the initial period of the experiment (180 d) vide table 7b. It then declined to the baseline value of 1.13 ± 0.17 ng/ml one month after the end of the trial (300 d), which was significantly (P<0.05) lower than 270 d value of 1.46 ± 0.25 ng/ml (table 7b).

The fluctuating upstream trend seen in animals of G I was repeated in the serum T_3 concentration of animals of G II (table 7a), which was 1.04 ± 0.08 ng/ml on 180 d and hiked to the highest value of 1.38 ± 0.18 ng/ml on 270 d,

which was significantly ($P<0.05$) higher than the 180 d value of 1.04 ± 0.08 ng/ml (table 7b). The serum T_3 concentration then declined, as in animals of G I, to the basal value of 1.13 ± 0.14 ng/ml on 300 d which was significantly ($P<0.05$) lower than the 270 d value of 1.38 ± 0.18 ng/ml (table 7b).

Statistically significant ($P<0.05$) differences were observed in the T_3 concentration, on comparing between animals of G I and G II, on days 195, 210 and 225. The serum T_3 concentrations of animals of G I and G II were 1.38 ± 0.21 ng/ml and 0.92 ± 0.12 ng/ml on 195 days of age, 1.19 ± 0.16 ng/ml and 0.96 ± 0.05 ng/ml on 210 days of age and were 1.42 ± 0.19 ng/ml and 1.17 ± 0.18 ng/ml on 225 days of age respectively (table 7a).

4.5.3 T_4:T_3 ratio

A highly fluctuating trend was quite evident in the thyroxine: triiodothyronine (T_4:T_3) ratio of animals of both control (G I) and experimental (G II) groups during the study period of 180 to 270 days of age.

The ratio of T_4:T_3 of animals of G I elicited a fluctuating trend with a decreasing fashion (table 7a) and reached the value of 28.88± 4.52 at the end of the trial (270 d) which was significantly ($P<0.05$) lower when compared to a ratio of 36.10 ± 4.43 at the start of the trial (180 d) vide table 7b. The ratio was then elevated to a value of 33.07 ± 9.58 one month after the end of the trial (300 d) vide table 7b.

The ratio of T_4:T_3 of animals of G II also had the fluctuating trend, as in animals of G I (table 7a) and attained the highest value of 42.44 ± 4.03 on 210 d

and declined to the lowest value of 31.94 ± 4.57 on 270 d, which was significantly ($P<0.05$) lower when compared to the 180 d value of 35.69 ± 7.53 (table 7b). Unlike animals of G I, the T_4:T_3 ratio of animals of G II had a value of 32.83 ± 6.06 on 300 d (table 7b).

All comparisons between animals of G I and G II employing student *t* test revealed statistically significant ($P<0.05$) differences with respect to T_4:T_3 ratios on days 195, 210 and 225. The corresponding T_4:T_3 ratios of animals of G I and G II groups were 28.48 ± 10.24 and 39.14 ± 1.54 on 195 days of age, 36.69 ± 4.36 and 42.44 ± 4.03 on 210 days of age and were 24.65 ± 2.35 and 32.29 ± 7.18 on 225 days of age respectively (table 7a).

4.5.4 Insulin

A definite increasing trend in the insulin concentration of animals of both control (G I) and experimental (G II) groups (fig.8) was quite evident during the experimental period from 180 d to 270 d.

Serum insulin concentration of animals fed with formaldehyde untreated soyabean meal had the lowest value of 7.53 ± 2.55 μU/ml at initial phase of the experiment (180 d) and hiked to the highest value of 11.12 ± 3.80 μU/ml at the end of the trial of 270 d (table 7a). The concentration then decreased to the basal value of 7.33 ± 2.35 μU/ml one month after the end of the trial (300 d) vide table 7b.

The increasing trend observed in animals of G I was reflected in the insulin concentration of calves of G II which reached the highest value of 12.00

Table 7a. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum hormonal traits of crossbred calves (n=6)

Age in Days	Thyroxine (T_4) (ng/ml)			Triiodothyronine (T_3) (ng/ml)			T_4:T_3 ratio			Insulin (µU/ml)		
	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value	Group I	Group II	't' value
180	37.38 ± 3.91	37.06 ± 7.96	0.09^{NS}	1.04 ± 0.11	1.04 ± 0.08	0.00^{NS}	36.10 ± 4.43	35.69 ± 7.53	0.11^{NS}	7.53 ± 2.55	7.80 ± 3.28	0.16^{NS}
195	37.91 ± 9.45	36.10 ± 4.35	0.42^{NS}	1.38 ± 0.21	0.92 ± 0.12	4.53*	28.48 ± 10.24	39.14 ± 1.54	2.52*	9.83 ± 1.82	7.83 ± 2.57	1.55^{NS}
210	42.97 ± 3.91	40.58 ± 3.65	1.09^{NS}	1.19 ± 0.16	0.96 ± 0.05	3.43*	36.69 ± 4.36	42.44 ± 4.03	2.37*	10.63 ± 3.07	8.33 ± 1.57	1.63^{NS}
225	34.72 ± 3.84	36.80 ± 5.19	0.79^{NS}	1.42 ± 0.19	1.17 ± 0.18	2.33*	24.65 ± 2.35	32.29 ± 7.18	2.48*	10.08 ± 2.60	8.83 ± 2.54	0.84^{NS}
240	38.36 ± 4.99	34.45 ± 3.03	1.64^{NS}	1.29 ± 0.17	1.21 ± 0.11	1.02^{NS}	30.18 ± 5.56	28.62 ± 2.47	0.63^{NS}	10.35 ± 4.02	10.00 ± 1.74	0.20^{NS}
255	42.98 ± 3.10	43.69 ± 3.53	0.37^{NS}	1.42 ± 0.33	1.23 ± 0.20	1.18^{NS}	31.56 ± 6.57	35.68 ± 6.26	1.11^{NS}	10.20 ± 4.10	11.33 ± 2.47	0.58^{NS}
270	41.44 ± 5.16	43.38 ± 3.53	0.76^{NS}	1.46 ± 0.25	1.38 ± 0.18	0.67^{NS}	28.88 ± 4.52	31.94 ± 4.57	1.16^{NS}	11.12 ± 3.80	12.00 ± 3.57	0.42^{NS}

NS – Non-significant

* - Significant at ($p<0.05$)

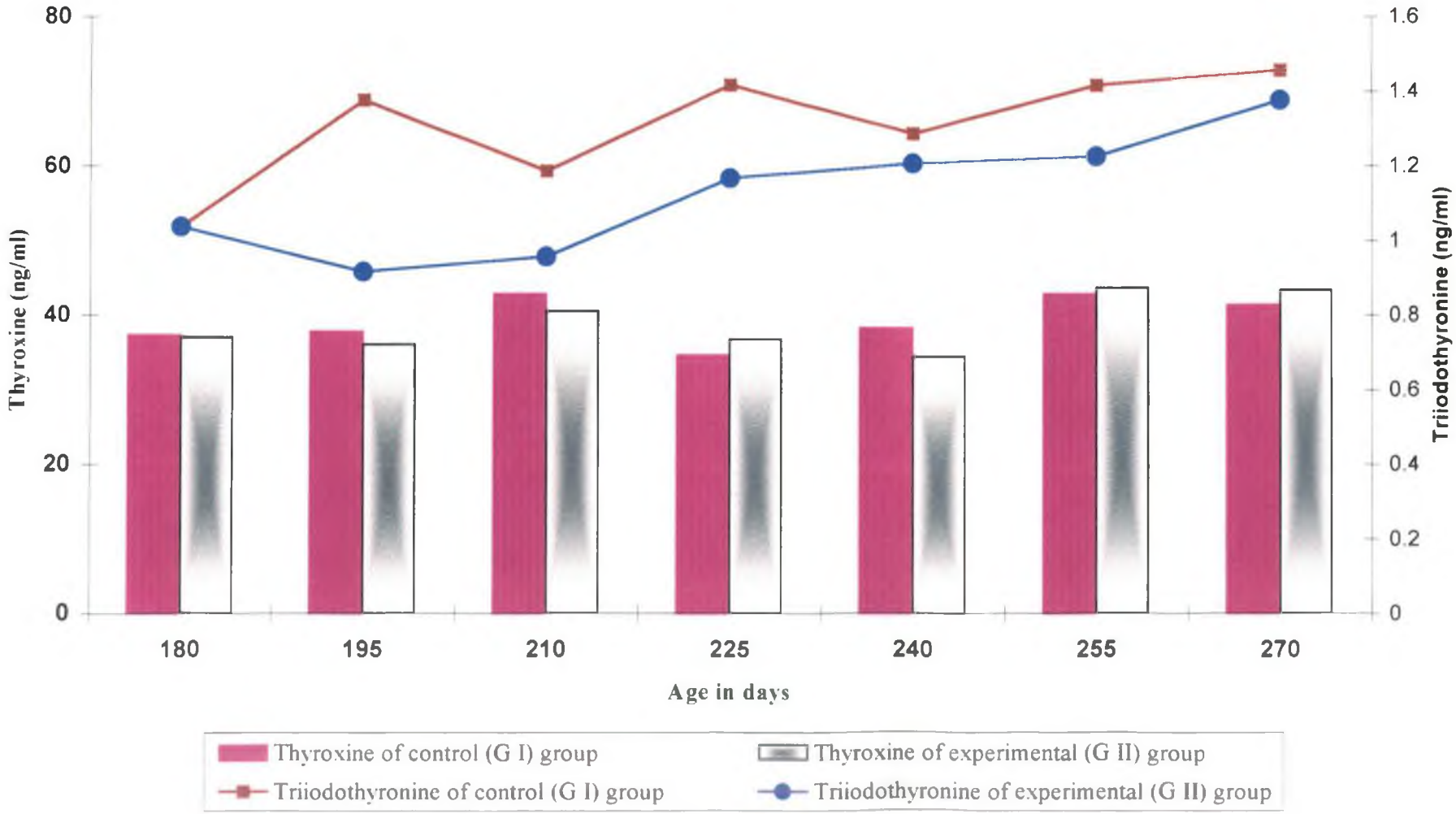
Table 7b. Effect of supplementation of the concentrate ration with untreated and formaldehyde treated soyabean meal on serum hormonal traits of crossbred calves (n=6)

Sl. No.	Sampling periods	Thyroxine (T_4) (ng/ml)		Triiodothyronine (T_3) (ng/ml)		T_4:T_3 ratio		Insulin (µU/ml)	
		Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
A	Before the start of the trial (180 d)	37.38 ± 3.91	37.06 ± 7.96	1.04 ± 0.11	1.04 ± 0.08	36.10 ± 4.43	35.69 ± 7.53	7.53 ± 2.55	7.80 ± 3.28
B	At the end of the trial (270 d)	41.44 ± 5.16	43.38 ± 3.53	1.46 ± 0.25	1.38 ± 0.18	28.88 ± 4.52	31.94 ± 4.57	11.12 ± 3.80	12.00 ± 3.57
C	One month after the end of the trial (300 d)	36.29 ± 8.18	36.39 ± 3.94	1.13 ± 0.17	1.13 ± 0.14	33.07 ± 9.58	32.83 ± 6.06	7.33 ± 2.35	7.42 ± 2.59
't' value for A vs B		1.47^{NS}	2.31^{*}	3.98^{*}	5.29^{*}	2.55^{*}	2.43^{*}	2.19^{NS}	2.83^{NS}
't' value for A vs C		0.28^{NS}	0.31^{NS}	1.37^{NS}	1.52^{NS}	0.78^{NS}	1.03^{NS}	0.25^{NS}	0.18^{NS}
't' value for B vs C		1.20^{NS}	3.68^{*}	2.71^{*}	2.68^{*}	0.82^{NS}	0.39^{NS}	1.62^{NS}	2.34^{NS}

NS – Non-significant

* - Significant at ($p<0.05$)

Fig.10. Effect of supplementation of the concentate ration with untreated and formaldehyde treated soyabean meal on serum thyroxine and triiodothyronine concentrations of crossbred calves



± 3.57 μU/ml on 270 d when compared to the lowest value of 7.80 ± 3.28 μU/ml on 180 d (table 7a). Similar to the trend in animals of G I, the insulin concentration of calves of G II reverted to a baseline value of 7.42 ± 2.59 on 300 d (table 7b).

Serum insulin concentration did not reveal any statistically significant difference between animals of G I and G II on employing the student *t* test (table 7a).

4.6 Fractionation of serum protein by Agarose Gel Electrophoresis

The electrophoretic separation of serum proteins of animals of control (G I) and experimental (G II) groups on days 180, 270 and 300 are given in plates 1 and 2. The lanes of the electrophoretic separation of serum proteins of calves of both groups follow their respective trend as in the biochemical evaluation of serum proteins.

Plate 1. Electrophoretic separation of serum proteins of animals of control group (G I) on days 180, 270 and 300

Lane 1 – on 180 d

Lane 2 – on 270 d

Lane 3 – on 300 d

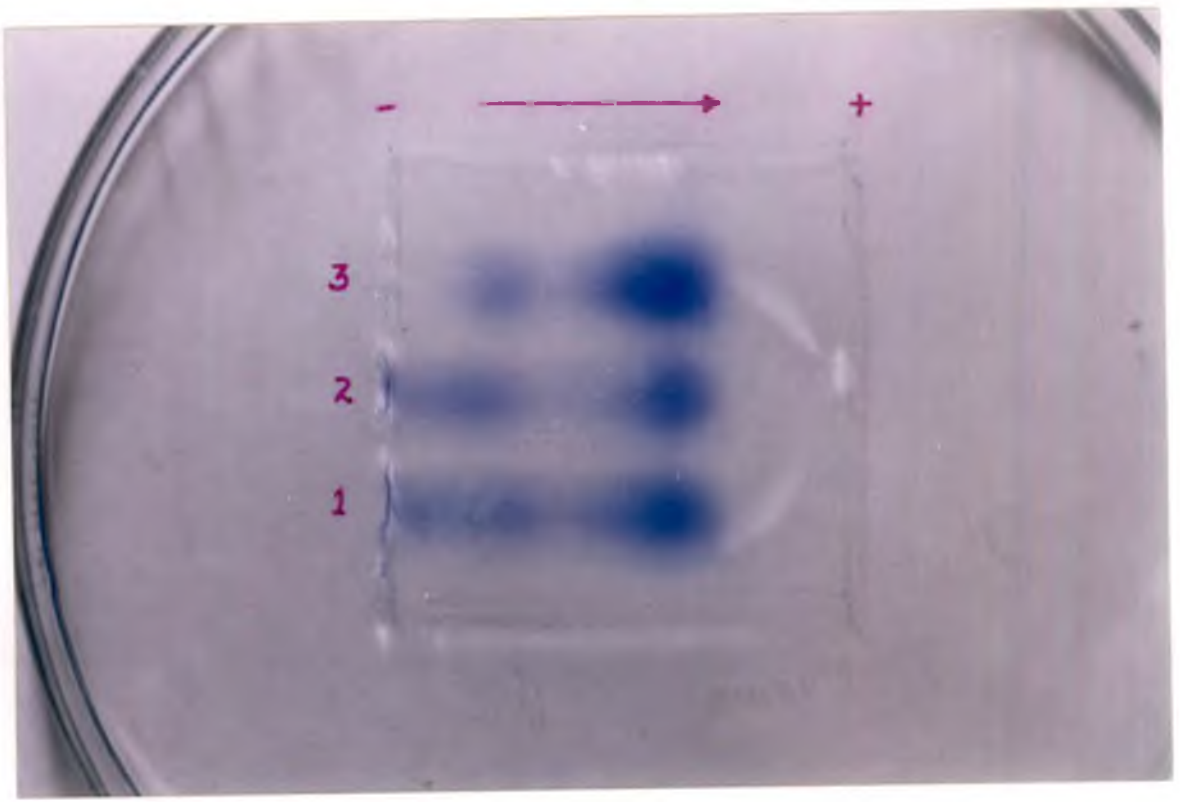
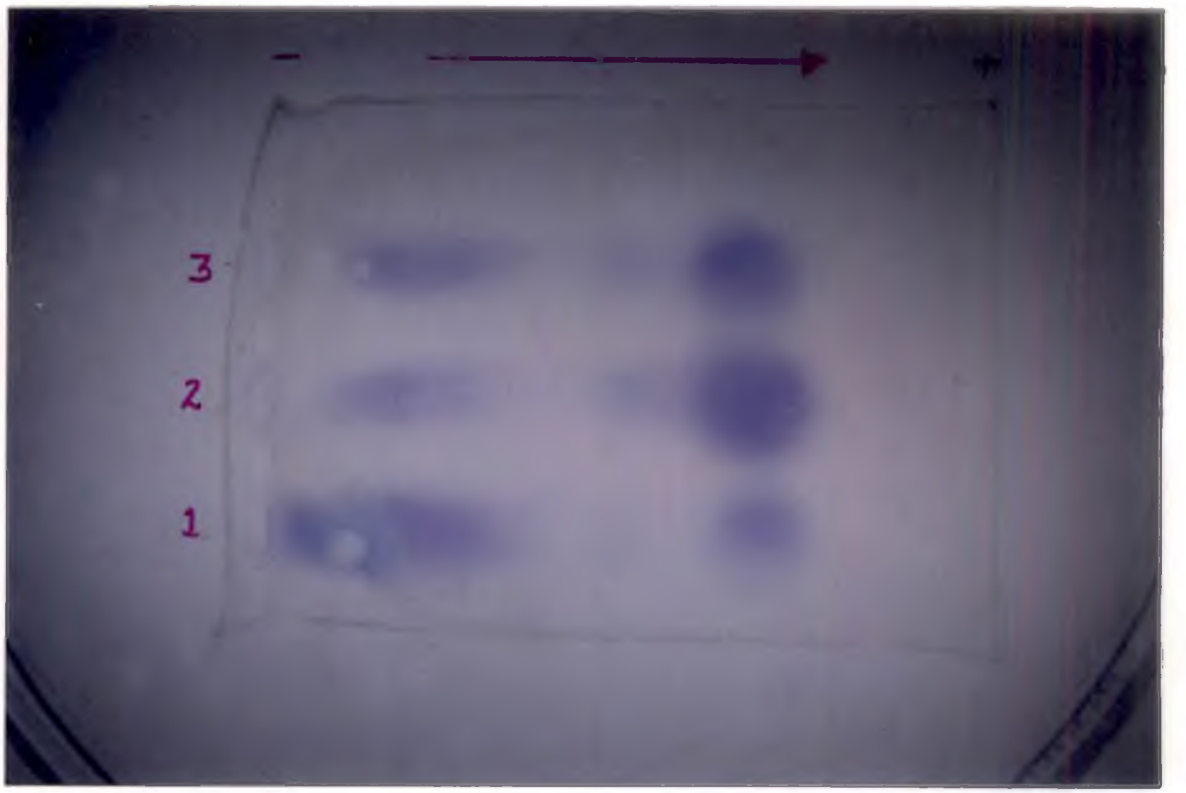

-
+
3
2
1

Plate 2. Electrophoretic separation of serum proteins of animals of experimental group (G II) on days 180, 270 and 300

Lane 1 – on 180 d

Lane 2 – on 270 d

Lane 3 – on 300 d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5. DISCUSSION

5.1 Composition and crude protein content of the ration

In the present study, the concentrate ration incorporated with formaldehyde treated soyabean meal was found to contain more crude protein content than the concentrate ration with untreated soyabean meal and hence, the animals of experimental group (G II) were receiving higher crude protein content of 30.33 per cent compared to 29.67 per cent crude protein fed to animals of control group (G I) vide table 1b, the reason for this variation remains unknown. This is in close accordance with the findings of Srivastava and Mani (1991), who reported that formaldehyde treated soyabean protein tended to have more crude protein than untreated one (24.3% vs 23.8%) on proximate analysis. Similarly, increased crude protein content of formaldehyde treated mustard cake over untreated cake was described by Giri and Dass (1993). Hence, the intention of providing more dietary protein to the experimental animals has been met with, as the standard concentrate mixture offered in the farm contained only 22 per cent crude protein (ICAR standards).

5.2 Body weight gain of animals

There was an increased gain in body weights of calves of experimental group (G II) fed with concentrate ration treated with formaldehyde than body weights of calves of control group (G I) reared on higher crude protein content (formaldehyde untreated) and the results revealed the beneficial effects of

formaldehyde treated soyabean meal possessed over the untreated meal. The calves of G II recorded more daily body weight gain per animal when compared to the animals of G I (352.731 g/day/animal vs 327.460 g/day/animal) vide table 2. Kumar and Walli (1994) in crossbred calves and Giri and Dass (1993) in buffalo calves supported these results of the present study. Mehta and Srivastava (2000) suggested that crossbred calves fed with formaldehyde treated grain exhibited an increased efficiency of nitrogen utilization, which could be due to greater utilization of amino acids from dietary protein.

Providing increased amount of dietary proteins over and above the requirement of growing animals is likely to increase the growth rate and hence, increased body weight gain. In animals of experimental group (G II), formaldehyde treated soyabean meal would have escaped ruminal microbial degradation, leading to an increased availability of amino acids for intestinal absorption. The elevated systemic availability of post-ruminal amino acids in animals of G II might have resulted in better growth process via certain hormones, viz., insulin, thyroid hormones and growth hormone; all anabolic in nature, in bringing about comparatively more body mass than the animals of control group (G I) fed with untreated soyabean meal. Moreover, increased dry matter intake encountered with animals of G II would have contributed for the increased growth rate. Though, the daily body weight gain of animals of G II were comparatively higher than that of animals of G I, the latter possessed higher mean body weights both at the initial and at the end of the trial (fig. 1).

5.3 Haematological parameters

The haematological parameters viz., haemoglobin (Hb) content, total erythrocyte (RBC) count, total leucocyte (WBC) count and volume of packed red blood corpuscles (VPRC) of crossbred calves of control (G I) and experimental (G II) groups showed continuously increasing trend, (table 3a). Normally, the products of digestion are absorbed and finally reached the circulation of animals and hence the haematological as well as biochemical analysis of blood will reveal the impact of dietary inclusion of various feed ingredients. Haematological changes are closely linked with systemic needs of animals, which vary with the growing period.

The increased Hb content, elevated RBC and WBC counts with respect to increased digestible crude protein content of the diet were well established by Patel *et al.* (1972), Hussain *et al.* (1975) and Pande and Shukla (1979). The upstream trend in the above mentioned three haematological parameters may probably be associated with an increased body weight gain of the animals, as increased body mass is likely in need of more haematological constituents to cope up with its normal turn-over and wear-out processes.

The growing crossbred calves when provided with increased amount of protein either as concentrate ration with untreated soyabean meal or concentrate ration with formaldehyde treated soyabean meal in the present investigation might have stimulated haemopoietic stem cells and hepatic system to produce

of haemoglobin present in erythrocytes and MCHC, indicating the average percentage of erythrocytic volume that the haemoglobin occupies.

Changes in the erythrocytic indices between animals of G I and G II were non-significant, reflecting the non-significant changes observed in the three haematological parameters viz., haemoglobin concentration, total RBC count and VPRC, utilized to calculate the erythrocytic indices. One month after the end of the trial, the erythrocytic indices again revealed non-significant differences, which could also be attributed to the same reason mentioned above (table 3b).

5.4 Biochemical parameters

5.4.1 Serum protein profile

The nitrogenous compounds of plasma encompass all those organic and inorganic nitrogen containing compounds of blood such as the proteins and nucleic acids, the smaller molecular weight compounds such as glutathione, urea and creatinine and inorganic compounds such as nitrate and ammonia. Regulation of protein metabolism is an intricate mechanism, encompassing wider areas of dietary intake, body's turn-over, growth, physiological drains seen in parturition and lactation, which are mainly controlled by hormones. Generally, increased protein intake in growing animals is associated with anabolic processes.

5.4.1.1 Total

Even though a significantly increasing trend in serum total protein content of animals of control (G I) and experimental (G II) groups was observed in the present investigation, this increase on comparison between groups was non-significant (table 4a). However, significant differences exist in serum total protein content of both the groups even one month after the end of the trial when compared to their respective values at the start of the trial (table 4b).

High dietary protein intake, over and above the requirement in growing animals is likely to augment positive nitrogen balance of the body, resulting in a significantly higher protein constituents in the blood. This was ascribed by Rekwot *et al.* (1989) and Tiwari and Yadava (1990) in Zebu bulls and buffalo calves respectively. Pachauri *et al.* (1999) reported an increased serum protein value of 7.38 g% in female crossbred calves fed with a concentrate ration replaced with 1% formaldehyde treated groundnut cake when compared to a value of 6.77 g% in control animals with no replacement. These values are closely in consonance with present findings (7.70 g% vs 6.5 g%).

These results might be attributed to the fact that when the requirements for the net tissue synthesis is effectively met by the post-ruminal supplies of "limiting" amino acids through varying amount of rumen undegradable protein (UDP) in the form of raw soyabean meal (containing about 31% UDP) and formaldehyde treated soyabean meal (having 70% UDP), the overall protein

status of the animal is likely to be increased. The non-significant differences in serum total protein content observed between both groups might be associated with the notion that calves of both groups would have probably been fed with an excess crude protein than the requirement, which have resulted in maximum growth rate.

5.4.1.2 Albumin

In the present investigation, a slow progressing trend in the serum albumin concentration was observed in both the groups (table 4a). Albumin, the most prominent of serum proteins, is synthesized by liver and is catabolized in all metabolically active tissues. Albumin is a major labile storage reservoir of proteins as well as a transporter of its constituent amino acids. It is the most osmotically active plasma protein and function as a general binder and transport protein.

The finding in the present study is in close agreement with that reported by Rekwot *et al.* (1989) who disclosed that Zebu bulls and their Friesian crosses showed higher serum albumin concentration with high protein diet (14.45% crude protein) than the low protein diet (8.5% crude protein) fed animals. However, Jordan and Swanson (1979) found that dietary crude protein content did not affect serum total protein or albumin concentration of high producing dairy cows.

There appears to be a direct correlation between albumin turn-over and body size (Kaneko *et al.* 1997). The continually increasing body size of the

calves of both groups in the present trial would have relied on more albumin content for the increased osmolarity as well as transportation related functions. This increased demand for amino acids turn-over might have been effectively met with increased synthesis of plasma protein as albumin following an elevated protein supply to the animals. The increase in albumin concentration can also be justified by the elevated demand for osmotic pressure regulating components by increased body size.

This elevated albumin status at the end of the trial (270 d) reached a baseline level in animals of experimental (G II) group but remained elevated in animals of control (G I) group, even one month after the end of the trial (table 4b). This may be related with the increased body size of the calves of G I group over that of calves of G II group and hence, an increased requirement for albumin.

5.4.1.3 Globulin

Serum globulin concentration, as calculated by subtracting serum albumin portion from total protein content, showed different trend in animals of control (G I) and experimental (G II) groups (table 4a). In general, it seemed to follow an increasing fashion reaching highest values at the end of trial. Serum globulins are generally fractionated into α_1 globulins, α_2 globulins, β globulins and γ-globulins or immunoglobulins. The former three are glycoproteins in nature and synthesized by the liver, while the latter is synthesized by plasma cells (derived from B lympholytes) in response to antigenic stimulation.

Globulins generally carry out transportation and immunological defence functions.

The findings of the present experiment are in close consonance with those reported by Rekwot *et al.* (1989) who reported a significantly ($P<0.05$) higher α-2 globulin, γ-globulin and total globulin in Zebu bulls and their Friesian crosses fed with high protein ration than those on low protein ration. The increased dietary protein availability in the body system, through its stimulatory effect, increased hepatic protein synthesis of α and β portions of globulins and hence, increasing trend in globulin concentration of both groups was observed.

Globulin concentration remained elevated at one month after the end of the trial in both the groups, since the total protein content remained elevated, resulting in an elevated globulin concentration (table 4b). The significantly ($P<0.05$) higher globulin concentration in animals of G II at 300 d of age in comparison to that at 180 d of age could also be attributed to the above reason.

5.4.1.4 Albumin:Globulin (A:G) ratio

The A:G ratio revealed fluctuating trends in both the groups during trial period of 180 d to 270 d of age (table 4a). The A:G ratio of both the groups during the period from 195 d to 255 d is more leaning towards albumin concentration revealing significantly increased albumin production. Moreover, the fluctuations are within the normal range, ruling out the probable reason of any disease condition. The reduction of A:G ratio to the baseline value in both

the groups, one month after the end of the trial (table 4b) may be suggestive of reduction of albumin concentration in relation to the serum total protein content.

5.4.2 Serum lipid profile

Lipid metabolism is usually highlighted in heifers, pregnant and lactating animals where the importance of steroid hormones is quite evident. In growing animals, lipid metabolism tends to be down regulated as protein anabolism overwhelms the lipid anabolism. However, the lipid constituents estimated in the present investigation revealed an overall increasing trend. Lipids in blood plasma of ruminants may arise from intestinal absorption of dietary lipids, mobilization of lipids from storage in adipose tissue, or synthetic processes. Most plasma lipids are present as chylomicrons and other density lipoproteins. In addition, non-esterified or free fatty acids are transported as a complex of fatty acid with albumin. Two of the most important functions of lipids are energy storage and membrane structure. Dietary protein has been demonstrated to regulate the rate of lipogenesis and has also been shown to control rate of fatty acid synthesis and related activities of a number of enzymes (Swensen and Reece, 1996).

5.4.2.1 Total lipids

Increasing trend in the concentration of total lipids of calves of control (G I) and experimental (G II) groups was evident during the trial period (table 5a). The lipid anabolism observed in the present study may be correlated with

the increasing body size. The bioconversion of dietary proteins and other essential nutrients to lipids in the liver is seen when it is continually and excessively supplied with such substrates and so formed lipids can act as energy reserve. Growth in terms of increase in size and number of cellular components probably requires more membrane constituents, 40 per cent of which are essentially lipids in nature. Hence, increased total lipids concentration in animals with increasing body size may be attributed to the elevated requirements of lipids for membrane constituents and as energy reserve.

The present findings did not agree with reports of Hutjens and Schultz (1971). They concluded that differences in plasma lipids were small between the formaldehyde treated and untreated soybeans (high fat content with 80% unsaturated fatty acids) fed groups. The present study, aimed at evaluating the effect of the increased protein intake on lipid metabolism, differs from them in the fact that the solvent extracted soybean meal contained a maximum fat content of 1.5%. One month after the end of the trial, concentration of total lipids in serum reached to baseline values (table 5b) in both the groups justifying the decreased demand by the decelerated body gain (fig. 1).

5.4.2.2 Cholesterol

Cholesterol concentration of both the groups revealed an upstream tendency during the trial period (table 5a). Findings of Hutjenz and Schultz (1971) also supported the present observation as they reported serum

cholesterol concentration of 14.6 g/dl in group fed with formaldehyde treated soybeans against 12.6 g/dl in untreated group. Park (1985), while evaluating the influence of dietary protein on blood cholesterol of growing calves reported that a reciprocal relationship existed between the dietary protein level and plasma cholesterol concentration. He also suggested that the amount of dietary protein acted as a regulator of plasma cholesterol by exerting its influence upon rates of cholesterologenesis but the underlying mechanism remained unknown.

The increasing trend in cholesterol concentration in the present study could also be associated with the increased demand for the cholesterol esters and free cholesterol for incorporating into cell membranes when body size was increased. However, the serum cholesterol concentration remained elevated one month after the end of the trial (table 5b).

5.4.2.3 Triglycerides

The present study revealed a pertinent upstream trend in serum triglycerides concentration of both the groups during the trial period (table 5a). Triglycerides or triacylglycerols are the most significant group of lipids from the stand point of energy metabolism of animals. They may be provided by diet or may be synthesized from non-lipid sources, largely in the liver, adipose tissue and the lactating mammary gland. Their concentration can vary greatly, depending on dietary intake. The findings of the present study alienate from those made by Hutjens and Schultz (1971) in lactating goats. They found a significantly ($P<0.05$) decreased concentration of triglycerides in goats

supplemented with untreated and formaldehyde treated soybeans in comparison to that of unsupplemented goats.

It could be thought that an increased availability of post-ruminal amino acids would have stimulated an increased insulin release, which would have increased glucose uptake by adipose tissue as well as fatty acid re-esterification with glycerol. This promoted adipose tissue to release lesser free fatty acids to the blood with concurrent increase in triacylglycerol formation. The decreased triglycerides concentration to a baseline value, one month after the end of the trial (table 5b) further justifies the above mechanism. i.e., when an increased protein supply was withheld, decreased synthesis of triglycerides was resulted.

5.4.2.4 Non-esterified fatty acids (NEFA)

The present trial revealed a fluctuating with an overall decreasing trend in the concentration of NEFA in both the groups (table 5a). Fatty acids are released to plasma from adipose triglycerides through the action of hormone-sensitive lipase. The fatty acids then physically bind to plasma albumin and are transported to the heart, skeletal muscles, liver and other tissues for oxidation or conversion to other lipids. Since the turn-over rate of NEFA are extremely rapid (1-3 min.), their serum concentrations are normally low. The finding of the present study closely agrees with those of Bunting *et al.* (1996). They reported that calves fed with undegradable protein as roasted soybeans tended to have a smaller percentage decrease in concentrations of NEFA.

Biosynthesis of long-chain fatty acids is under hormonal, nutritional and metabolic influences. The increased provision of proteins to the growing calves through untreated as well as formaldehyde treated soyabean meal would have satisfied protein as well as energy requirements, over and above the normal requirement. Hence, lipolysis in adipose tissues to release free fatty acids so as to meet the energy requirement might not arise in calves with positive nitrogen balance. Moreover, increased insulin release associated with increased post-ruminal amino acids availability would have minimized the lipolysis in adipose tissue through the inhibition of hormone-sensitive lipase and at the sametime, promoted triglycerides formation (table 5b).

A significantly ($P<0.05$) elevated NEFA concentration, one month after the end of the trial in animals of experimental group (G II) vide table 5b may be explained by the fact that when increased provision of dietary protein was withheld, associated beneficial effects like increased insulin release and fat sparing action were forcefully withdrawn leading to lipolysis to meet energy requirements and hence, release of free fatty acids to blood (table 5b). This would have additionally reduced the triglycerides formation (table 5b) because of reduced fatty acid synthesis and increased lipolysis.

5.4.3 Blood glucose level (BGL)

A pertinent increasing trend was evident in BGL of crossbred calves of both control (G I) and experimental (G II) groups during the trial period (table 6a). This might be attributed to the fact that increasing body size in terms of

withdrawal of high dietary protein intake, the energy demands might have been reduced and comparatively reduced BGL may be sufficient to meet the demands.

5.4.4. Serum urea nitrogen / blood urea nitrogen (BUN)

A continuous rising trend in serum urea nitrogen concentration in both the groups during the experimental period was noticed (table 6a), which was in close association with the reports made by Srivastava and Mani (1995) and McCormick *et al.* (1999). However, a significant decrease in plasma urea nitrogen concentration in response to intake of undegradable protein supplementation was reported by Broderick *et al.* (1993) and Chen *et al.* (2002).

In the present study, increased dietary protein intake through untreated as well as formaldehyde treated soyabean meal would have provided more availability of proteins for metabolism, over and above the requirements for growth. Hence, the excess might have been deaminated and converted into urea for excretion. The decrease of serum urea nitrogen concentration to a baseline value, one month after the end of the trial, may also be attributed to the above reason. When excess dietary protein intake was withdrawn and standard concentrate ration was followed, there seemed no excess protein available and hence, further increase in the serum urea nitrogen was upheld (table 6b).

size and number of cells would be requiring more energy to carry out their metabolic functions, which could be satisfied by glucose. Normal blood sugar level (BGL) of body is maintained by the interplay of several hormones including insulin, glucagon, epinephrine, glucocorticoids and thyroid hormones. Under post-absorptive conditions, BGL varies considerably and the variability is often related to nutritive state (Swenson and Reece, 1996). The liver occupies a central position in the regulatory mechanism of BGL because it supplies and also removes glucose from the system. The major direction of liver glucose metabolism is directed towards supplying rather than using glucose.

The insulin release stimulated by the increased protein intake could not have reduced the rising BGL in both the groups and because the increased BGL observed in the present trial was also within the normal range of 60-80 mg/dl (Swenson and Reece, 1996). Increased insulin release might have been utilized for protein anabolism and triglycerides synthesis (Guerino *et al.*, 1991). The elevated BGL would have been utilized for meeting energy demands during growth process in calves. The reports of Palmquist *et al.* (1993) alienated from the present findings. Based on a study in cows fed with diets high in fat and undegradable protein, they reported that blood glucose concentrations were not influenced by diet.

The lowered BGL, one month after the end of the trial (300 d), in both the groups (table 6b) may also be explained by the relationship of body size and energy demands. Since body weight gain increase was slowed down due to

withdrawal of high dietary protein intake, the energy demands might have been reduced and comparatively reduced BGL may be sufficient to meet the demands.

5.4.4. Serum urea nitrogen / blood urea nitrogen (BUN)

A continuous rising trend in serum urea nitrogen concentration in both the groups during the experimental period was noticed (table 6a), which was in close association with the reports made by Srivastava and Mani (1995) and McCormick *et al.* (1999). However, a significant decrease in plasma urea nitrogen concentration in response to intake of undegradable protein supplementation was reported by Broderick *et al.* (1993) and Chen *et al.* (2002).

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5.4.5 Creatinine

A definite decreasing trend was observed in the serum creatinine concentrations of animals of both control (G I) and experimental (G II) groups during the experimental period (table 6a). Most of the creatinine excreted originates from endogenous creatine, which is synthesized in liver, circulates in plasma and later taken up by muscle to store energy in the form of phospho-creatine. Creatine is the sole precursor of creatinine and the conversion is a non-enzymatic, irreversible process. The quantity of creatinine formed each day depends on the total body content of creatine, which in turn depends on dietary intake, rate of synthesis of creatine and muscle mass. Factors influencing muscle mass such as disease, tissue wasting and physical training, all of which can affect the size of the creatine pool and thus, the daily production of creatinine (Kaneko *et al.*, 1997).

The trend in present study may be attributed to less damage to the muscle mass of animals during the trial period accomplished by increasing protein accretion caused by increased dietary protein intake. In other words, the tissue wasting might be minimum during the period of increased dietary protein intake. However, one month after the end of the trial, the serum creatinine content was again marginally raised without significant variation between groups and between periods within a group (table 6b). There was least wasting of tissue (muscle) during the experimental period and even after one month after feeding of high protein diet.

5.4.6 Bilirubin

The present investigation revealed a pertinent decreasing trend in both the groups during the experimental period (table 6a) and a slight increase (table 6b) in the concentration of serum bilirubin, one month after the end of the trial. Bilirubin is a yellow pigment produced by enzymatic degradation of heme, part of haemoglobin. Approximately 80% of the bilirubin produced is derived from the degradation of heme of haemoglobin of erythrocytes. Degradation of heme from other sources (eg. myoglobin, cytochromes, peroxidase and catalase) accounts for the remaining bilirubin production. Liver contains large quantities of microsomal cytochromes (P450 and b5 system) and is the most important non-erythroid bilirubin source. The unconjugated bilirubin formed elsewhere is bound to albumin in plasma and then taken by the liver and converted to polar, conjugates such as bilirubin diglucuronide which are excreted through biliary system.

5.5 Serum hormonal traits

Evaluation of key hormones viz., thyroxine, triiodothyronine and insulin, during the growing period of animals in the present study revealed an increasing trend in crossbred calves of both control (G I) and experimental (G II) groups.

5.5.1 Thyroxine (T_4)

A fluctuating, yet an increasing trend was clearly evident in thyroxine (T_4) concentration of crossbred calves of both control (G I) and experimental

(G II) groups in the present trial (table 7a). The thyroid hormones viz., thyroxine (T_4) and triiodothyronine (T_3) control metabolic processes, growth and differentiation, reproduction and lactation in all animals. Thyroid hormones stimulate the basal metabolic rate via the metabolism of carbohydrates, lipids and proteins (Hoch, 1974). The actions are mediated by increasing the activities of specific enzymes that contribute to oxygen consumption. Of the two iodinated thyronines, thyroxine (T_4) is predominant in calves.

Increasing body size resulting from the intake of higher dietary protein would be requiring more oxygen for consumption by the growing cells. These oxidative metabolic demands resulted in an increased synthesis and release of thyroid hormones from the thyroid gland. The fluctuations observed in the present trial were inevitable as they are concerned with the episodic release. Moreover, growth is the result of interplay of critical hormones like growth hormone, insulin and thyroid hormones. Besides, T_4 and T_3 appear to stimulate growth hormone release (Swenson and Reece, 1996) resulting in an enhanced growth and these hormones are important in cell differentiation.

In addition, increasing body size, associated with increased size and number of the cells of thyroid gland, would have thereby got the capacity to trap more iodide and synthesize more thyroxine. This also justified the decrease in T_4 concentration of both the groups, one month after the end of the trial, when increased dietary protein supply and associated increase in body size were diminished vide table 7b (Barash *et al.*, 1998).

5.5.2 Triiodothyronine (T_3)

A fluctuating but a progressing trend in triiodothyronine (T_3) concentration was exhibited by animals of both control (G I) and experimental (G II) groups during the experimental period (table 7a). Most of the circulating T_3 is derived from peripheral deiodination of thyroxine. The *in vivo* potency of T_3 is about three times that of thyroxine. The metabolic requirement for oxygen consumption by growing calves would have warranted an increased synthesis and release of T_3 from thyroid gland, as in the case of thyroxine. There found significantly ($P<0.05$) lower concentrations of T_3 during the period from 195 to 225 days of age in G II animals, in comparison to G I animals (table 7a). This might be attributed to the comparatively reduced body weight of G II animals during the same period when compared to G I animals (fig.1). However, at the end of the trial, the T_3 concentration of G I animals was found to be marginally higher than that of G II animals, reaffirming the increased body size of G I animals over G II animals on 270 d of age (fig. 1).

One month after the end of the trial, T_3 concentrations of crossbred calves of both groups reached a baseline value (table 7b). As protein supplementation was withheld, the rapid increase in body size was curtailed and hence, basal line concentrations of T_3 and thyroxine would have been sufficient to meet the metabolic oxygen demands.

5.5.3 T_4 : T_3 ratio

A highly fluctuating trend was observed in T_4 :T_3 ratios of calves of both groups during the trial period (table 7a). Similar to the progress of T_3 concentration, T_4 : T_3 ratio of animals of experimental group (G II) revealed significant ($P<0.05$) differences over that of animals of control group (G I) during the period from 195 d to 225 d of age. Comparatively lower concentration of T_4 and T_3 of animals of G II during that period over that of animals of G I could be the probable reason, signifying the relationship between body size and weight and secretion of thyroid hormones. The increase of T_4 : T_3 ratio in both groups, one month after the end of the trial, would explain the effect of withdrawal of increased dietary protein.

5.5.4 Insulin

There found to be a definite increasing trend in insulin concentrations of crossbred calves of both control (G I) and experimental (G II) groups during the experimental period (table 7a). Insulin, a two-chain peptide molecule, has equally important effects on protein and fat metabolism as does with carbohydrate metabolism. Insulin increases the transport of most amino acids into muscle, stimulates protein synthesis and inhibits protein catabolism. It stimulates hepatic synthesis of fatty acids and insulin is thus, considered as a hormone that promotes anabolism (Swenson and Reece, 1996).

Increased jugular insulin concentrations have been measured in sheep fed with formaldehyde treated casein (Faichney and Weston, 1971) and in

sheep abomasally infused with casein (Barry et al., 1982). Further, Waghorn *et al.* (1987) found that insulin concentrations were higher in growing sheep fed with high protein diets. The present study results were in close agreement with those made by Guerino *et al.* (1991) who demonstrated a positive relationship between amino acid absorption and pancreatic insulin secretion. They also concluded that the increases in insulin secretion caused by increased protein intake were consistent with insulin's ability to promote nutrient storage by animal tissue.

The upstream trend in insulin concentration might be similarly correlated with the increased insulin secretion by pancreas caused by increased dietary protein intake through untreated as well as formaldehyde treated soyabean meal, signifying the anabolic effect of insulin on protein accretion. Moreover, the decrease of insulin concentrations to a baseline value, one month after the end of the trial, in both groups (table 7b), further signified the impact of increased dietary protein intake on pancreatic insulin secretion.

Plasma concentration of a hormone is the net result of secretion into the circulatory system minus clearance from the blood. (Guerino *et al.*, 1991). The basal concentrations of circulating hormones in ruminants fluctuate due to a number of intrinsic factors such as episodic hormone release and diurnal rhythm. Other factors such as ambient temperature, diet and feed intake can also influence basal hormone concentrations.

5.6 Fractionation of serum protein by Agarose Gel Electrophoresis

The electrophoretic separation of serum proteins of calves of control (G I) and experimental (G II) groups on days 180, 270 and 300 are in close agreement with the evaluation of serum proteins by biochemical methods on those respective days (plates 1 and 2).

Summary

6. SUMMARY

The present study was conducted in twelve numbers of female crossbred calves of similar age and size of the University Livestock Farm, College of Veterinary and Animal Sciences, Mannuthy, for a period of 90 days (180 to 270 d of age). The animals were maintained under standard managemental conditions with *ad libitum* provision of drinking water and roughage. The animals were divided into two groups, Group I (control) and II (experimental), with six calves in each group. One third of the concentrate ration of Group I animals was replaced with raw soyabean meal (solvent extracted) while, in Group II animals, 1% formaldehyde treated soyabean meal (rumen undegradable protein) was used for replacement.

Body weight of all the animals were recorded fortnightly during the entire period of study. Blood samples were collected with and without anticoagulant from all animals of both groups at the initial state of experiment (180 d), thereafter on every 14 days and one month after the end of the experiment (300 d). The blood samples were collected with anticoagulant (heparin) and analysed for blood glucose level (BGL) and haematological parameters like haemoglobin content, total erythrocyte and total leucocyte counts and volume of packed red blood corpuscles using standard procedures and erythrocytic indices were calculated. The serum was separated from whole blood without anticoagulant and subjected for the estimation of biochemical

parameters such as concentrations of total protein, albumin, globulin, total lipids, cholesterol, triglycerides, non-esterified fatty acids (NEFA), urea nitrogen, creatinine and bilirubin employing commercial kits. Hormonal traits like thyroxine (T_4), triiodothyronine (T_3) and insulin were also estimated using radioimmuno assays.

On analysing the proximate principles of various concentrates, it was found that both the groups received increased dietary protein intake and particularly animals of Group II, through protected proteins. Animals of Group II recorded a higher daily weight gain of 352.731 g/animal/day in comparison to 327.460 g/animal/day of animals of Group I.

All the haematological parameters screened revealed a persistently increasing trend, with non-significant differences between groups. Serum concentrations of total protein and albumin pursued an increasing trend in both groups with non-significant differences between them. Serum globulin concentration revealed fluctuating yet an increasing trend in calves of both groups. A continuous upstream trend was evident in calves of both groups in BGL, concentrations of serum total lipids, cholesterol, triglycerides and serum urea nitrogen during the trial period. A decreasing trend in the serum NEFA status of both the groups was evident. Serum creatinine and bilirubin levels of both groups showed a decreasing trend. Concentrations of hormones viz., T4, T3 and insulin exhibited an increasing trend in calves of both the groups during the entire period of experiment.

The upstream trend in the haematological constituents may be associated with an increased body weight gain of the animals and hence, with an increased protein requirement. The serum protein profile proved the elevated protein status of the animals with positive nitrogen balance. Moreover, there appears to be a direct correlation between albumin turn-over and body size. The globulin concentration of both the groups suggested an increased hepatic synthesis of α and β portions of globulins.

The lipid profile of calves of both groups indicated an increased requirement of these constituents as membrane constituents and as energy reserve, for the build up of body size. Increased insulin release stimulated by increased availability of post-ruminal amino acids would have favoured an increased triglycerides synthesis. Decreasing trend in serum NEFA status of both the groups signified the reduced lipolysis for energy purpose with an increased dietary protein intake.

The rising BGL observed in calves of both the groups elaborated the increased energy demands for the enhanced growth process. An increasing trend in serum urea nitrogen might be due to deamination and conversion into urea of excess protein in calves of both groups fed over and above the actual requirement. The increased metabolic demand for oxygen associated with the increased body size might have resulted in an increased synthesis and release of thyroid hormones, with significant ($p<0.05$) differences between groups. The fluctuating T4:T3 ratios in both the groups corresponded to the episodic release

of the hormones. The insulin concentration demonstrated a positive relationship between amino acid absorption and insulin release and might have promoted protein anabolism resulting in an increased body size with positive nitrogen balance.

It could be summarized that the provision of increased dietary protein to the growing crossbred calves either through raw soyabean meal or through rumen protected soyabean meal by treating with formaldehyde will bring about protein anabolism with positive nitrogen balance, with an added advantage. It is also revealed that rumen protected protein prepared by one per cent formaldehyde treatment not only favoured cost reduction in preparation of bypass protein but also improved the growth performance of crossbred calves.

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EVALUATION OF CERTAIN METABOLIC AND HAEMATOLOGICAL PARAMETERS IN CROSSBRED CALVES FED WITH RUMEN UNDEGRADABLE SOYABEAN MEAL

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ABSTRACT OF A THESIS

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ABSTRACT

Protecting ruminal feed proteins by formaldehyde treatment decreased their solubility and degradation in the rumen, eventually resulted in an increased availability of post-ruminal amino acids and this could be exploited for enhancing the growth rate of young ruminants as crossbred calves. Post natal growth in ruminants is chiefly influenced by the metabolic hormones, whose secretions are, in turn, regulated by the circulating levels of critical amino acids. Hence, the objective of the present study was to determine and correlate the levels of certain metabolic hormones and other related haematological cum biochemical parameters in growing crossbred calves, fed with concentrate ration supplemented with untreated and formaldehyde treated soyabean meal.

Twelve numbers of female crossbred calves of six months of age of the University Livestock Farm, College of Veterinary and Animal Sciences, Mannuthy, were divided in to two groups, viz., Group I and Group II, with six calves in each group. One third of the quantity of concentrate ration to be given to each animal was replaced by raw soyabean meal (solvent extracted) in animals of Group I and 1% formaldehyde treated soyabean meal in animals of Group II, during the experimental period of 90 days (180 to 270 d of age). Drinking water and roughage were provided *ad libitum*. All the animals were maintained under standard managemental conditions.

Fortnightly body weight of all the animals were recorded during the entire period of study. Blood samples were collected from all animals of both groups at the initial phase of experiment (180 d), thereafter on every 14 days as well as one month after the end of the experiment (300 d). The blood samples were analysed for blood glucose level (BGL) and haematological parameters like haemoglobin content, total erythrocyte count and total leucocyte count, volume of packed red blood corpuscles and erythrocytic indices using standard procedures. The serum was subjected for the estimation of biochemical parameters such as concentrations of total protein, albumin, globulin, total lipids, cholesterol, triglycerides, non-esterified fatty acids (NEFA), urea nitrogen, creatinine and bilirubin employing commercial kits. Hormonal traits like thyroxine (T_4), triiodothyronine (T_3) and insulin were also evaluated using radioimmuno assays.

On analysing the proximate principles of various concentrates, it was found that both the groups received increased dietary protein intake and particularly animals of Group II, through protected proteins. Animals of Group II recorded a higher daily weight gain of 352.731 g/animal/day in comparison to 327.460 g/animal/day of animals of Group I.

The haematological parameters screened revealed a persistently increasing trend, with non-significant differences between groups. This upstream trend may be associated with an increased body weight gain of the animals and hence, with an increased requirement. Serum concentrations of

total protein and albumin pursued an increasing trend in both groups with non-significant differences between them. This signified the elevated protein status of the animals with positive nitrogen balance. Moreover, there appears to be a direct correlation between albumin turn-over and body size (Kaneko *et al.*, 1997). Serum globulin concentration revealed fluctuating yet an increasing trend in calves of both groups, suggesting an increased hepatic synthesis of α and β portions of globulins. A continuous upstream trend was evident in serum total lipids, cholesterol content and triglycerides concentration of calves of both the groups during the trial period, indicating an increased requirement of these constituents as membrane constituents and as energy reserve, for the build up of body size and weight. Increased insulin release stimulated by increased availability of post ruminal amino acids would have favoured an increased triglycerides synthesis. Decreasing trend in serum NEFA status of both the groups signified the reduced lipolysis for energy purpose with increased dietary protein intake.

Blood glucose level of calves of both groups revealed a pertinent increasing trend, elaborating the increased energy demands for the enhanced growth process. An increasing trend in serum urea nitrogen concentrations observed in calves of both the groups might be due to deamination and conversion into urea of excess protein in calves of both groups fed over and above the actual requirement. Serum levels of creatinine and bilirubin in both groups of animals showed a decreasing trend which may be attributed to the fine balance in their level by increased protein supplementation. Serum

concentrations of hormones as T_4, T_3 and insulin exhibited an increasing trend in calves of both the groups. Increasing trend in insulin concentration demonstrated a positive relationship between amino acid absorption and insulin release and might have promoted protein anabolism resulting in an increased body size with positive nitrogen balance. The increased metabolic demand for oxygen associated with the increased body size might have resulted in an increased synthesis and release of thyroid hormones, with significant ($p<0.05$) differences between groups. The fluctuating T_4:T_3 ratios in both the groups corresponded to the episodic release of the hormones.

Results of the present study substantiate that increased dietary proteins had brought about elevated protein anabolism and associated haematological, biochemical and hormonal changes in growing crossbred calves offered with concentrate ration supplemented with rumen protected soyabean meal and this was certainly having an advantage in the crossbred calves, over the calves provided with increased rumen degradable proteins. Since protein supplements are generally the most expensive ingredients in ruminant rations, thus there is an interest in maximizing their utilization.