

DISCUSSION

5.1. Body weight gain :

The requirement of protein and amino acids in growing animals have been determined mostly by the estimation of total body weight gain. The method is considered quite valid one because, normal growth in general, can be correlated with the tissue protein, which is the primary constituent of the body. However, it has also been shown that the body composition differs with dietary alterations, and in some cases the weight gain is more dependent on deposition of fat than increase of body protein. In the present investigation the average total gain in body weight by advocating 3 grade of ration were 36.46 ± 0.67 (group I), 24.0 ± 1 (group II) and $34.6 \pm 1g$ (group III) respectively. The gain in body weight was however observed significantly lower in case of animal belongs to group II maintained in ration with 10% protein diet in comparison to other 2 groups (group II and III) which were maintained with higher protein percentage in their rations (Fig.20). The gain in body weight was quite similar in group I and III, and statistically there is no significant difference observed in between these two groups. However, there is significant difference ($P/0.5$) in body weight of group II with that of group I and III.

Muramatsu and Ashida (1962) observed that the growth rate in rats increases as the level of casein supplemented in the diet increased from 0-25%. The same group of workers in 1961 also observed that the body weight of albino rat increases as the level of soyabean protein increased in the diet, the maximum growth achieved by soya bean protein was reported to be 17% or more. Westerfeld et al. (1962) has reported that the growth rate of chicks were increased along with the increased protein level in their diet. In human being, Gopalan et al. (1984) has reported that in child the protein malnutrition is largely responsible for growth performance of the body as a whole, and also mental function and learning capacity appears to be affected in protein malnutrition during early stage of childhood. Extensive work has been carried out with low and high protein diet in domesticated animals. Warth and Migra (1927) found that the calves with average body weight of 212 pound grew at the rate of 1.09 pound per day when fed with crude protein 11.4%, whereas Morrison (1936) found the growth rate is 0.60 found with such crude protein. Bedrak et al. (1966) reported that in heifers given less protein, gain less body weight along with loss of vitality and roughness of hair.

Sharma et al. (1973) studied growth rate in Tharparkar calves with 2 levels of protein ration raised over a period 137 days. The average body weight growth was found

to be more satisfactory in animal with DCP and TDN level at the average of Morrison's recommendation while in the other groups which received 24.5% less DCP were found to be poor in growth rate. Similar work also conducted in buffalo heifer calves (Mudgal and Siviah, 1982) with three level of protein in 48 growing buffalo calves divided in 6(six) groups. The overall results showed that the better growth and feed utilization can be achieved in buffalo heifers at higher protein level 100 to 110% level.

The gain in body weight, in albino rat observed in the present study is quite in agreement with the finding of the other workers in different species. The present study has showed that the 10% protein level in the diet is quite inadequate to achieve proper growth, resulting malnutrition in albino rat.

5.2. Gain in Liver weight :

It is well documented that due to starvation in animals (rat) the weight of the liver falls rapidly and recently it was worked out that malnutrition leads to the improper function of liver which also affect total weight of the liver. In the present investigation the average weight of the liver were found to be 8.22 ± 0.39 (group I), 6.07 ± 0.18 (group II) and 7.69 ± 0.39 (group III) respectively, at

the end of 25 days of feeding period. The highest weight of the liver was observed in control group which is followed by group III with 20% protein level. The lower liver weight was observed in animal with 10% protein level. There is significant difference in between the group II with group I & III. Similar work was also been reported by Albanese in 1963. With low level of protein resulting decrease in liver weight. Murmatsu and Ashida (1961) reported that the body weight and liver weight increases along with the level of casein fed for 15 days in rat, the maximum body growth and liver weight was observed by feeding 25% of casein diet. The results of the present investigation is quite in agreement with the aforesaid workers.

5.3. Blood haemoglobin :

The interrelation between the protein reserve of the body, haemoglobin and the circulating plasma protein has been worked out extensively by whipple (1948). A dietary deficiency of protein may interfere with Hb protein. In the present investigation the mean haemoglobin percentage values in different groups were 14.5 ± 0.45 (group I), 11.6 ± 0.38 (group II) and 15.1 ± 0.36 (group III) as shown in table C.1. The lower haemoglobin percentage was observed in animals belongs to group II and is quite likely due to the lower level of dietary protein (10%) fed during the experiment. It is

quite evident from the table C.1. that the three levels of protein have got some effect on the haemoglobin content of the blood. These findings also in conformity those of Weech et al. (1937) in dog, Kyer and Bethal (1938) in rat and Orten and Orten (1940) on growing rats. Many workers have also found the differences in haemoglobin content with age and strain of same species. Hardy (1967) observed that the level of haemoglobin in winter strain of rat with different age group i.e., 6 week to 18 months differed in between 13.6 gm% to 17.8 gm% of haemoglobin respectively. Similar work conducted by Weisse et al. (1974) observed that the haemoglobin percentage values at different age groups i.e., 6, 14, 34 weeks were 14.7 gm, 16.9 gm and 17.0 gm per 100 ml. In farm animal Mudgal (1963) reported increased level of blood haemoglobin in Sahiwal, Red Sindhi and Tharparkar heifers while feeding high level of protein in their ration. However, Rathee and Yadava (1970) failed to observed any significant change in haemoglobin concentration in the blood of Haryana calves by feeding different protein levels in their diets. These findings from available literatures confirm to some extent that the level of protein in the diet is quite related with the haemoglobin concentration in the blood. The observation of this study reveals that 10% protein in rat has lowered the haemoglobin value, in comparison to the other two groups where the protein content is much higher. Lower level of Hb. in blood indicated the malnutrition in the group fed

with 10% protein. Various workers have already observed a reduction in haemoglobin content in malnourished animals.

5.4. Blood protein :

Considerable progress has been made to exploit the function of protein in biological sciences. Nutritionally scientists are much more concerned about the quantity and quality of the protein present in the diet. Deficiency of protein in animals and in human leads to different type of malfunction in the body as a whole. Presently the percentage of protein in the diet is often questioned in respect of different species of animals. In the present study it has been observed that by giving only 10% of protein in the diet leads to certain pathological syndromes in rat. The total protein content in the blood of these animals were 5.8 ± 0.19 gm% (group II), whereas in other two groups the mean values of the blood protein were quite high 7.1 ± 0.25 (group I) and 7.13 ± 0.24 (group III) and without any difference. The last two groups were supplemented with normal ration and 20% protein respectively. Many works have been conducted to see the effect of dietary protein in different level, which causes the lowering of endogenous protein in the body. Wainio et al. (1953) has reported that the total protein in the blood became low in pair fed albino rat in comparison to the animals which were fed ad-libitum. In some species Heimer et al. (1972)

and Kozma et al. (1969) have observed that the total protein in rat was 6.3 ± 0.23 and 7.17 ± 0.39 gm% with normal diet, which was quite comparable with the values of the present investigation. The estimation of total protein in blood plasma is a reflection of the nutritive status of the animal. The less protein in the diet as observed by Addis et al. (1936), Kosterlitz (1947) and Harrison and Long (1945) in a series of experiments by feeding protein free diets, the animal mostly suffered from depletion in liver protein. Melnick et al. (1936) and Cox and Mueller (1944) suggested that the various food proteins differ qualitatively in ability to promote synthesis of plasma protein. Madden et al. (1938) demonstrated that few dietary proteins favour the production of plasma albumin and also globulin. In dog, Chow et al., (1945) reported that protein depletion in diet effects the plasma protein concentration in blood within 5 to 8 weeks of feeding protein free diet, the plasma protein value drops down from 6.8 to 5 gm/100 ml. Hegsted et al. (1946) reported that the total protein and its fractions tend to decrease when low protein of all vegetable diets were fed in human adults. The values of total protein noted in this present investigation are thus quite similar to the results of other workers. The present investigation has reflected that the 10% protein level in the diet leads to lowering of circulating protein in the plasma, which leads to appearance of malnutrition syndrome in albino rat.

5.5. Tissue Enzymes :

Enzymologists has shown that the activity of various enzymes in animal tissue are affected by alteration in the nutritional regime from that of normal. Many workers have investigated that any alteration of protein in the diet is a major cause which affects the activities of many tissue enzymes. Addis et al. (1936) has demonstrated that the animals either fasted or subjected to a diet varying in protein content exhibit drastic change in liver composition. Similar observations in adult rats and in chicken were also reported respectively by Ross and Batt (1956), Stanier (1957), Summer and Fisher (1960). It has been reported that due to protein malnutrition, the liver tissue is very prone to exhibit any change, in general liver enzymes are quite labile and different enzymes responded to protein deficiencies in different ways. The level of some liver enzymes may be increased and others may decreased. It has also been suggested that in protein deficiency there may not be any alteration or there may be little or no change. Since enzymes are protein in nature they are expected to participate in the dynamic equilibrium of protein metabolism. Enzymes like hepatic Xanthine oxidase, catalase, arginase, alkaline phosphatase and many others are very susceptible to dietary intake of little/no protein. In the present investigation study the effect of variation in dietary protein level on liver catalase, hepatic transaminase, gamma amylase has been observed.

5.6. Enzyme Catalase :

It has been reported that the catalase activity in liver tissue is more in comparison to kidney tissue. In the present study the level of liver catalase were 1.73 ± 0.13 (group I), 1.91 ± 0.04 (group II) and 1.70 ± 0.13 (group III) respectively and it has been observed that the catalase activity is slightly higher (not significant) in animals which were maintained with 10% protein (group II) in comparison to group I and group III. From the findings it can be suggested that the low level of protein increases the catalase activity. Similar observation was also made by Mukherjee and Sarker (1958) that the enzyme catalase and alkaline phosphatase level increases due to protein deficiency in human patient suffering from malnutrition. They have also stated that the increase level of catalase in such cases may also be due to a higher content of iron in the liver. The estimation of liver iron in the present study was however not done. Miller (1948) has reported that the reduction in the amount of catalase during starvation is due to decrease rate of synthesis and increase rate of break down in liver tissue of rat. It may be that increase rate of hepatic tissue break down has caused an increased level hepatic catalase as observed in the present investigation. Catalase synthesis and destruction were also studied under the condition of protein

deficiency in diet. The results of the present investigation is quite in agreement with the aforesaid workers. Further it is well established fact that depletion of dietary protein leads to impairment in the activity of the liver catalase, which is quite relevant with 10% protein of the present investigation.

5.7. Gamma Amylase :

The enzyme amylase generally designated as a group of enzymes that catalyse the hydrolytic degradation of starch and glycogen (Searchy, 1969). In recent years, it has been clearly shown that the liver cell of various animals contain amylase and numbers of extra parenchymatic tissue are also capable of synthesising amylase. The values of liver Gamma amylase recorded in this study were 44.4 ± 0.92 (group I), 24.13 ± 1.77 (group II) and 45.23 ± 1.95 (group III) respectively the lowest value of gamma amylase being in the animals fed with 10% protein, whereas, in other two groups the values were quite high and without any difference. The lowest value of gamma amylase may be due to impairment in the synthesis of the enzyme in the protein deficiency state. In human subject Lehndorff (1961) reported that the amylase value reduced in patient suffering from Kwashiorkor, Albanese (1963) opined that protein depletion decrease the total activity of various liver enzymes because the average

weight of the liver in depleted animals is quite lower than that of the animal fed ad-libitum. From the result of the present investigation it appears that the 10% protein which reduces the liver weight has also affected the lowering of liver enzymes

5.8. Enzymes Transaminase :

It was not until 1938, that the enzymatic transamination was first described as a new concept involving intermolecular transfer of an alpha amino group from an amino acid to an alpha keto acid without formation of free ammonia. This set of enzyme occurs in most organism and various type of enzyme have been characterized in bacteria as well as in animal tissue. Normally all most all of the transaminase are localized within the cells and relatively small amount of enzymes circulate in plasma. Heart and liver contain the highest concentration of GOT, where as much less located in lung and spleen. It is of special interest that the hepatic tissue possess the highest content of GOT, with respect to both transaminase, only relatively minute amount of enzyme present in serum under normal condition. Boyde and Latner (1962) has found that when the saline extract of human liver, heart and kidney is subjected to electrophoresis, two slow and fast moving bands can be seen. Similar observation has also been made using rat liver extract by Boyde (1961). It has been

reported earlier that there is change in the liver enzymes due to malnutrition in most of the species. The fluctuation of GOT and GPT level in liver tissue is one of the diagnostic tool to assess the function of liver. In the present investigation it has been noted that the activities of these two enzymes have seriously been affected due to malnutrition. The average value of GOT in the liver tissue of animals maintained in 10% protein level was 126 ± 5.76 I.U./mg of liver tissue protein whereas the average value of GOT are 226 ± 22.19 and 226 ± 4.79 in group I and III respectively. From this study it is quite relevant that the liver GOT increases progressively along with increase of protein intake. Similar observation has also been made by Muramatsu and Ashida (1962) by advocating high level of casein in the diet. Such increase level of GOT was also reported in animal suffering from liver diseases by Tomicki (1961) and Robert (1968).

The trend of GPT is almost similar which has been observed in GOT. The average mean values were 75.73 ± 4.46 (group I), 50.93 ± 5.63 (group II), 77.73 ± 4.71 (group III). It has been observed that the low value recorded in group II is due to malnutrition with 10% protein diet, whereas in other two cases (group I and III) the values are quite high and identical. Similar trend in GPT has been reported by

Muramatsu and Ashida (1962), they observed that the κ level of GPT increases when the animal were fed higher level of casein. Many workers, (Summers and Fisher, 1960, Akehi, 1957, Soberson and Sanchez, 1961) have reported that the level of GPT increases in protein depleted animals is might be due to increase of amino acid resulting from rapid destruction of tissue protein, which may result leakage of large amount of this enzyme outside the parenchyma (Cornelius, 1970). The high level of SGOT and SGPT in high protein diet could be attributed to increased gluconeogenesis.

The values of SGOT and SGPT in this present investigation is in agreement with those of Muramatsu and Ashida (1961). Further it is well established that the malnutrition with 10% protein in rat has significantly reduces the synthesis of SGOT and SGPT.

5.9. Thyroid hormone :

The activity of thyroid hormones are mostly reflected by oxidative metabolism, growth and differentiation of tissue. Recently it has been worked out that 99.98% of circulating thyroxine (T_4) and 99.9% of triiodothyronine (T_3) are bound to serum protein. In the blood essentially all T_4 and T_3 are transported bound to protein which make them metabolically inactive. Three (3) most important protein fractions which

bind the thyroid hormones are thyroxine binding globulin (TBG), thyroxine binding pre-albumin (TBPA) and albumin. Only minute fraction of T_4 about 0.02% and T_3 0.2% exist in free or unbound state and now it is currently thought that these free hormones are metabolically active moieties.

Recently, much more emphasis has been made to establish the correlation in between the endocrine function of the body in relation to malnutrition. Many workers has reported that malnutrition in animals and human beings has get some effect on the transportation of both peptides and steroid hormones. Murphy (1967) has extensively worked out the biological significance of the hormones carrier proteins, and Muga et al. (1967) has reported that the level of such proteins rises quite significantly during pregnancy. Many workers reported that the thyroid function decreases in malnutrition. Cochran et al. (1964) noted low serum PBI, Bear et al. (1966) reported low uptake of radio iodine, Davidson and Chopra (1979) observed that there is significant lowering of T_3 level due to low carbohydrate content in the diet. Chopra (1981) found that due to protein deficient diet there is decrease in serum total thyroxine level but the free thyroxine is not much affected. Lowering of T_3 and T_4 in malnourished animals is also reported by Larsen (1981). Few

workers have also tried to explain thyroid hypofunction in nutritional deficiency with low thyroid stimulating hormone (TSH) in malnutrition, it may be due to some disturbances in the built in safety devices for hormone secretion as well as synthesis.

In the present investigation as relevant from Table I.1 and Fig.24 that the T_3 level decreases significantly (0.63 ± 0.07 in group II) whereas in other two groups the levels of T_3 is quite appreciating, 1.30 ± 0.11 ng/ml in group I and 1.49 ± 0.16 in group III. In this finding it has been observed that the group II subjected to malnourish has reflected low level of T_3 . This lowering of T_3 in this group may be due to less content of binding protein in the system or it may be due to depressed activity of 5-diodinase enzyme activity which is responsible for T_4 to T_3 conversion in the tissue according to Davidson and Chopra (1979).

The level of thyroxine in this present investigation (Table J.1. Fig.24) was found to be somewhat in higher side 8.6 ± 2.9 $\mu\text{gm}/100$ ml in animals subjected to malnourish (group II). However this value is not significant. In other two groups the values were almost similar 7.16 ± 0.60 $\mu\text{gm}/100\text{ml}$ (group I), 7.6 ± 3.7 $\mu\text{gm}/100$ ml (group III) respectively. In case of thyroxine it is quite evident that the level of this hormone is not affected due to low level of protein. Recent

work from this laboratory Baruah et al. (1984) has also revealed that the different level of dietary protein has failed to affect the thyroxine hormone in kids (caprine) from 0-60 days of birth. In the present study the insignificant rise of T_4 in malnourished group may be due to high concentration of free T_4 in the system which may be due to less production of binding proteins. Moreover, in the method of estimation by RIA it has been adopted to estimate the total thyroxine (Bound T_4 + free T_4).

On the basis of above discussion it is very difficult to remark the present findings in malnourished diet with 10% protein. However, more than one mechanism are seemed to involve in lowering of T_3 and elevation of T_4 in case of malnourished group.