CHAPTER 7

Objective 6: Evaluation of the growth promoting ability and protein quality of mixes and biscuits in an animal model

Introduction

The quality of cereal proteins can be improved by mutual supplementation with legume proteins (Parpia and Swaminathan 1972). Addition of green leafy vegetables to cereals and pulses has also been shown to improve the protein quality of the mixtures (Talwalker and Patel 1970a, Talwalker and Patel 1970b, Adrian and Peyrot 1971, Naik et al 1978). Germination, an intermediate step in malting of grains, brings about quantitative and qualitative changes in proteins (Hwang and Bushuk 1973, Ganesh Kumar and Venkataraman 1978, Krishnamurthy and Venkataraman 1983, Sathe et al 1983, Duranti et al 1984). Thereby the quality of grain proteins improves as a result of increased digestibility of the proteins and because of the enhances that occur in free amino acids. In germinated grains increases in protein efficiency ratio (PER), digestibility coefficient (DC), net protein utilization (NPU) and relative nutritive value (RNV) have been reported by many investigators (Palmer et al 1973, El-Hag et al 1978, Khan and Ghafoor 1978, Wang and Fields 1978, Hamad and Fields 1979, Hasim and Fields 1979, Khader 1983). However, somewhat contradictory results have also been observed (Bates et al 1977) that the quality of grain proteins in terms of PER became inferior following germination.

Protein quality of cereal-pulse mixes with or without green leafy vegetables

Chatterjee and Abrol (1975) evaluated the protein quality of a mix of wheat and green gram in the ratio of 90:10. The chemical score of the product protein was 71% as against 53 and 47% of wheat and green gram, respectively. Similarly, a combination in the ratio of 80:20 of rice and green gram increased the chemical score of the mixture protein to 70% as against 57 and 47% of the 2 grains individually. Wheat, rice, maize and barley in combination with other legumes, viz., cowpea, lentil, pigeon pea and bengal gram were found to have higher biological values than the protein of the individual grains.

The protein quality of different combinations; wheat with bengal gram and rice with red gram or black gram was assessed by growth rate of rats and PER (Chandrasekharappa 1979). The value for PER of wheat protein at 10% level was 1.66 and that of bengal gram protein, 2.05. Supplementation of wheat with bengal gram to the extent of 12% by weight, significantly improved the growth rate as well as PER. Of the various blends of wheat and bengal gram tested, a blend of 60:40 protein ratio was found to give the highest PER of 2.50. No supplementary effect was observed when rice protein was supplemented with red gram or black gram.

Del-angel and Sotelo (1982) had determined the protein quality of 3 mixes having wheat to bengal gram protein ratio of 50:50 (mix 1) or 60:40 (mix 2) or 70:30 (mix 3). It was observed that supplementing wheat protein with bengal gram improved the

protein quality of the mixes as the PER value of mix 1 (1.55), 2 (1.35) or 3 (1.39) was higher than that of wheat alone (0.65). But the PER values of the 3 mixes were comparable inspite of increasing the amount of bengal gram protein in the wheat bengal gram mixes.

Jaya and Venkateraman (1980) had supplemented wheat and rice proteins with those of bengal gram and green gram respectively so that the ratio of cereal protein to pulse protein was 2:1. The PER values of the supplemented mixtures were higher than those of cereals and pulses separately. However, the supplementary effect was more pronounced when legumes were used with rice than with wheat.

Shehata and Fryer (1970) had evaluated protein quality of breads prepared from wheat flour containing 0, 5, 10, 15 or 20% bengal gram flour by a rat growth assay. The rats fed 20% bengal gram flour diet gained significantly more weight than those fed diets containing 0, 10 or 15% bengal gram flour. The PER values of 10 and 20% bengal gram flour diets were significantly higher than that of diet containing no bengal gram flour (1.46 and 1.56 Vs 1.18).

Juneja et al (1980) conducted an animal experiment to determine the PER of the wheat flour; triticale flour; wheat flour plus triticale flour (60:40); wheat flour plus triticale flour plus bengal gram flour (40:40:20) using skim milk powder as the reference protein. The results revealed that the PER value

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of the diet containing wheat flour plus triticale flour plus bengal gram flour was significantly higher than those of wheat flour, and wheat flour plus triticale flour diets. These findings indicated that the supplementation of bengal gram flour to a wheat plus triticale flour mixture helped to improve the biological value of the mixture.

Pushpamma and Devi (1979) had determined the nutritional quality of sorghum and legume based food mixtures. Sorghum and legume mixtures were prepared using red gram, black gram, bengal gram, green gram, cowpea, horse gram and soya bean. The sorghum and legumes were mixed so as to provide protein in the ratio of 2:1 from the 2 sources. The PER values of combinations between sorghum plus black gram or cowpea were significantly higher than those of the combinations of sorghum with bengal gram, soya bean, red gram, green gram or horse gram.

Narayanaswamy et al (1975) determined the supplementary relations between the proteins of ragi, jowar and cowpea. The PER values of ragi and cowpea proteins fed individually at 6% protein level were 1.29 and 2.05, respectively. When the 2 proteins were mixed in the ratio of 3:1 and 1.1 the values increased to 2.14 and 2.84, respectively. The PER values of jowar and cowpea proteins fed individually at 10% level were 1.09 and 2.18, respectively and increased to 1.79 and 2.16 when the proteins were mixed in the ratio of 3:1 and 1:1, respectively.

Talwalker and Patel (1970b) evaluated the jowar protein

supplemented with proteins of ambadi (Hibicus cinnabinus) and methi greens in terms of regeneration of liver and plasma proteins in 60 male rats of 150 g body weight. The diets were planned to provide 7% protein from jowar as the sole source of protein (Diet 1) or 5% jowar protein plus 2% ambadi protein (Diet 2), or plus 2% methi protein (Diet 3), or plus 2% casein protein (Diet 4). Six rats were sacrificed to get the initial values of liver and plasma proteins and the remaining rats were placed on a protein free diet for 3 weeks. Six rats were sacrificed to determine pre-experimental levels of liver and plasma proteins after their being on a protein free diet for 3 weeks. The remaining rats were divided into 4 groups of 12 rats each and were fed either jowar diet or jowar supplemented diets for 4 or 9 days. The hepatic protein content of rats on protein free diet decreased from 19.04 to 14.07 g/100 g and serum protein concentration from 6.68 to 4.29 g/dl. The rats fed Diet 1 for 4 days did not show any regeneration of liver proteins over the pre-experimental value of 14 g/100 g, on the contrary the protein content of liver had exhibited a decrease of 15% (from 14.07 to 12.00 g/100 g). However, with continued feeding on the same diet up to 9 days; the hepatic protein content showed an upward trend although the values still remained lower than the pre-experimental value (13.82 Vs 14.07 g/100 g). Such effects however, were not observed on serum protein levels as the levels remained higher than the pre-experimental levels after 4 days feeding trial but after 9 days, the serum protein levels were almost equal to the initial values. After 4 days of feeding jowar diet supplemented

with ambadi (Diet 2) or methi (Diet 3) or casein (Diet 4), the hepatic protein content increased by 17, 19 and 12% respectively. A further 10% increase in hepatic protein was recorded in response to feeding Diets 2 or 3 when the experiment was extended for another 5 days, with the result the hepatic protein contents of ambadi and methi supplemented groups were comparable to that of the initial value. But the hepatic protein of rats fed casein supplemented diet exhibited no further increase after 4 days and remained lower than the initial value (15.88 Vs 19.04 g/100 g). These data indicated that rats fed diets containing jowar supplemented with ambadi or methi had a higher capacity to regenerate liver proteins than diets containing only jowar or jowar supplemented with casein. The serum protein levels after 4 days of feeding Diets 2, 3 or 4 were above the pre-experimental values and by the end of the 9 days of feeding the levels were 26, 45 and 34% higher than the pre-experimental values but did not reach the normal values. These findings justified the use of green leafy vegetables for improving the quality of cereal protein. Talwalker and Patel (1970a) observed elevations in PER, BV and DC values of jowar protein (0.92, 79%, 90% respectively) when it was supplemented with ambadi (0.94, 81%, 92% respectively) and methi (1.54, 89%, 94% respectively) proteins.

The nutritional quality of rice fortified with dehydrated leaf powders of colocasia, coriander (Coriandrum sativum), mayalu (Basella rubra), radish and shepu (Peucedanum graveolens) was evaluated by Naik et al (1978). The diets provided 9.5%

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protein, the rice protein to leaf protein ratio was 70:30. It was observed that the PER values of rice protein diet supplemented with colocasia, coriander or shepu, were 72 to 77% of that of casein protein diet thereby suggesting the importance of green leafy vegetables in the enrichment of poor rice diet. However, the PER values of the mayalu and radish supplemented rice protein diets were somewhat low, as these were 50 and 64% of that of the casein protein diet.

Shukla and Sur (1978) had demonstrated that the cauliflower (Brassica oleracea var. botrytis) and radish leaf proteins had excellent supplementary value when given with rice and wheat proteins. The PER values of radish leaf and wheat (2.23) and cauliflower leaf and wheat (2.13) were significantly higher than the PER for wheat alone (1.80), thus establishing a significant supplementary effect of these leaf proteins to wheat protein. The PER values of radish leaf and rice (2.33) and cauliflower leaf and rice (2.48) were also higher than that of rice alone (2.22). The supplementation of rice with cauliflower leaf or radish leaf significantly increased the plasma protein levels while no such effects were observed when wheat was supplemented with leaf proteins. Supplementation of wheat with radish leaf, and of wheat and rice with cauliflower leaf, resulted in significantly higher hepatic protein levels than those resulting from feeding of rice or wheat alone. The authors recommended the use of radish and cauliflower leaves in human nutrition. Based on PER values, serum protein concentrations and hepatic

nitrogen contents, Sehgal et al (1975) had earlier demonstrated that the protein quality of wheat protein was improved when it was supplemented with sundried mustard, raya and spinach leaves.

Phansalkar et al (1957) determined the supplementary effect of a leafy vegetable on the protein efficiency ratios of cereals and pulses. The cereals used were bajra, jowar, rice and wheat; the pulses were bengal gram, black gram, green gram and red gram; and leafy vegetables were agathi (Sesbania grandiflora), amaranth, murungu (Moringa oleifera) and parpukeerai (Portulaca oleracea). It was observed that among the 4 leafy vegetables used as supplements to provide one-tenth of the total dietary protein (one percent from vegetable and 9% from either cereal or pulse), amaranth alone gave a significant supplementary effect. In the same study a 6:3:1 proportion of cereal:pulse:leafy vegetable protein was used for PER estimations. Addition of amaranth to wheat or bajra mixed with bengal gram or black gram or green gram or red gram markedly improved the PER values over those of the cereal-pulse combinations (7:3 protein ratio) but such effects were not observed with rice or jowar pulse combinations. Substitution of one part of the cereal or pulse protein by one part of the amaranth leaf protein did not significantly improve the hemoglobin or plasma protein levels. There were also practically no differences in the hemoglobin and plasma protein levels between the rats fed the cereal-pulse combinations and the cereal-pulse-leafy vegetable mixtures.

In contrast, Vaidehi (1983) had failed to demonstrate the supplementary effect of green leafy vegetables. The author had determined the protein quality of a nutri-mix in which 30 g of ragi malt was replaced by carrot (Daucus carota) greens powder. The nutri-mix contained 60 or 30 g of ragi malt, 15 g of defatted groundnut flour, 10 g of milk powder (full fat), 15 g of full fat soya flour and zero or 30 g carrot greens powder. The replacement of ragi malt by carrot leaf powder brought about no appreciable change in the PER as the PER value of the nutri-mix was 3.3 and of that with carrot leaf powder, 3.1.

Protein quality of germinated grains and malted mixes

Total and available lysine content : In 1976, Dalby and Tsai had observed increases in lysine content of wheat, barley, triticale, rye, oats and rice germinated for 5 days at 28°C. The increase in total lysine content ranged from 21 to 65% being highest in rye and lowest in rice. Wu and his co-workers (Wu and Wall 1980, Wu 1982, Wu 1983) have analysed various grains such as normal and high lysine sorghum, triticale and oats germinated for various days to observe the changes in their lysine contents. In 1980, Wu and Wall germinated normal and high lysine sorghum for one to 10 days at 22°C to 26°C. The authors observed that the total lysine content of normal lysine sorghum increased progressively from day 2 of the germination period. By day 10, the lysine content of the normal lysine sorghum had increased from 2.2 to 3.2 g/16g N exhibiting a 45% increase. On the other

hand, in high lysine sorghum, the increase in lysine content was progressive only until 7 days (from 3.0 to 7.8 g/16g N) of the germination period. Thereafter, the lysine content exhibited a decrease with the increase in germination period to 9 days (from 7.8 to 6.7 g/16g N). However, the lysine content of the 7 days germinated high lysine sorghum was higher than that of the 10 days germinated normal lysine sorghum (7.8 Vs 3.2 g/16g N) indicating that the increase in lysine content during germination might be determined by the initial lysine content of the grains.

In another study, Wu (1983) has made similar observations in oats which had been germinated for one to 8 days at 20°C to 26°C. The lysine content of the oats had not changed following 2 days of germination. Thenceforth, it began to increase and by the end of 8 days of germination the lysine content of oats had increased from 4.4 (initial value) to 5.3 g/16g N. However, in triticale germinated for one to 8 days at 20°C, Wu (1982) observed that the lysine content declined from its initial value of 3.5 to 3.3 g/16g N after one day of germination followed by a progressive increase of 79% until the end of the germination period (from 3.3 to 5.9 g/16g N).

Tsai et al (1975) germinated 2 varieties of maize for 5 days at 28 to 30°C. The authors observed that the total lysine content of both the varieties had increased by 87% (from 0.38 to 0.71%). Four years later, Ram et al (1979) also determined the lysine contents of 2 varieties of maize germinated for 5 days at 28±1°C. In both the varieties, there was no change in the lysine content

of one day germinated maize but it increased progressively from day 2 until day 5 of the germination period. In one variety of maize the total lysine increased by 164% (from 1.99 to 5.26%) while, in the other, it increased by 65% (from 2.65 to 4.37%). These data highlight the effects of varietal differences on lysine content of the germinated grains. But, the findings do not lend support to the point made earlier that probably the initial lysine content has a bearing on the lysine content of the germinated grains because the increase in lysine content of the variety of maize containing initially 1.99% lysine was higher than that containing 2.65% lysine (164 Vs 65%). It appears that more than one factor determine the changes in lysine content during germination of grains.

Studies have also been conducted to determine the lysine content of germinated legumes. Palmer et al (1973) had shown that the total lysine content of kidney beans germinated for 4 or 8 days at 18 to 23°C had increased from 5.9 to 6.1 g/16g N after 4 days of germination and declined to a level even below the initial value when the germination period was extended to 8 days (from 6.1 to 5.3 g/16g N). But the lysine content of soya bean germinated for 3 days at 50°C had not differed from the initial value of 6.0 g/16g N (Khader 1983). Likewise, in bengal gram, germinated for 16 h at 31°C, no marked changes were observed in the lysine content as compared to that of the raw bengal gram, 6.84 versus 6.56 g/16g N (Geervani and Theophilus 1980). On the other hand, Jaya and Venkataraman (1980) had

observed a decrease of about 8% (from 4.70 to 4.30 g/16g N) in lysine content of 48 h germinated bengal gram grains.

Increases in the available lysine content of germinated wheat, corn and sorghum grains have been reported. Hamad and Fields (1979) had demonstrated a highly significant increase in the available lysine content (from 23.3 to 114.9 mg/g N) in flour made from wheat which had been germinated for 5 days at 20°C. Earlier Wang and Fields (1978) germinated corn and sorghum for 3 to 4 days at 30°C. The available lysine content had increased by 151% (from 22.5 to 56.5 mg/g N) in germinated corn and by 122% (from 13.5 to 30.0 mg/g N) in germinated sorghum.

An increase in available lysine content has been observed in black gram germinated for 66hand at 25 to 27°C by Venugopal and Rama Rao (1978). The available lysine content increased from 4.10 to 5.21 g/16g N until 42 h of germination and thereafter, a steady decline was recorded until the end of the germination period. The decline was higher during 42 to 48 h than during 48 to 66 h of germination period (14 Vs 3%). But the available lysine content of 66 h germinated black gram was 7% higher than that of the ungerminated grains (4.37 Vs 4.10 g/16g N).

In contrast, decreases in available lysine content of germinated bengal gram have been reported by Geervani and Theophilus (1980) and Jaya and Venkataraman (1980). Geervani and Theophilus (1980) had reported a decrease in available lysine content of 16 h germinated bengal gram (from 85 to 75%) while

Jaya and Venkataraman (1980) demonstrated a decrease of about 9% (from 4.49 to 4.10 g/16g N) in 2 days germinated bengal gram.

In mixes prepared from 2 different varieties of malted ragi and green gram, Brandtzaeg et al (1981) had recorded a decrease in the lysine content from 368 (value of unmalted mix) to 341 and 321 mg/16g N.

Increases in the contents of other amino acids such as methionine and tryptophan in germinated grains have also been reported. Wang and Fields (1978) had reported that in 3 to 4 days germinated corn the available methionine content increased from the initial value of 9.0 to 45.0 mg/g N and available tryptophan content increased from 2.0 (initial value) to 13.0 mg/g N. Likewise, in 3 to 4 days germinated sorghum the available methionine content increased from 8.5 (initial value) to 15.0 mg/g N and available tryptophan content from 3.4 (initial value) to 19.5 mg/g N. Increases in tryptophan in 3 to 5 days germinated maize have also been reported (Tsai et al 1975, Dalby and Tsai 1976, Ram et al 1979). Wu and Wall (1980) had reported increases in methionine and cystine from 2.6 to 3.8 g/16g N in one day germinated sorghum.

In soya bean germinated for 6 to 7 days Wu and Fenton (1953) had observed that the tryptophan content had increased from 516 (ungerminated) to 604 mg/100 g. Later in 1983, Khader had also demonstrated a small increase in the tryptophan content of 3 days germinated soya bean. El-Shimi et al (1984) had shown that the

total tryptophan content had increased from 1.08 (ungerminated) to 1.25% in 4 days germinated fenugreek seeds. In 5 days germinated alfalfa seeds, total sulphur amino acids (from 227 to 319 mg/g N) were found to increase in comparison to those of the ungerminated seeds (Harrison and Vanderstoep 1984).

Relative nutritive value, protein efficiency ratio, biological value, net protein utilization and digestibility coefficient : Hamad and Fields (1979) had reported that relative nutritive values (RNV) of flours made from 5 days germinated wheat, barley and rice were significantly higher than that of the flour made from ungerminated grains. Also significant increases were observed in available lysine contents of flours made from germinated grains in comparison to that of those made from ungerminated grains. In 3 to 4 days germinated corn meal, similar increases in RNV (from 66.8 to 99.5%) and elevations in PER values (from 1.8 to 2.5) have been reported (Wang and Fields 1978). Likewise, increases in RNV from 55.3 to 62.9% and PER from 1.5 to 1.7 were observed in sorghum germinated for 3 to 4 days. The authors have attributed the increases in RNV and PER to increases in lysine, methionine and tryptophan contents.

Ram et al (1979) had determined the true digestibility (TD), BV, NPU and utilizable protein (UP) of one, 2 and 3 days germinated maize seeds. The authors observed that the TD values tended to decrease with the increase in germination time. But 14 to 16% increases in BV, NPU and UP values were observed

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following one day of germination period although further increase in germination period did not augument these values. The improvement in BV observed after one day of germination was attributed to better availability of lysine and tryptophan from storage proteins because increases in these amino acids were observed with increase in germination time.

Hemanalini et al (1980) had demonstrated that the growth promoting ability of germinated ragi was much higher than that of whole ragi as the weight gain (g/week) was 1.32 for germinated ragi diet fed rats and 0.61 for the whole ragi diet fed rats. However, no significant difference was observed in the PER values of germinated ragi (1.46) and whole ragi (1.41) proteins. The higher growth promoting value of germinated ragi was attributed to its higher B vitamin contents.

Geervani and Theophilus (1980) determined the protein quality of raw and 16 h germinated bengal gram using PER, BV and DC as the indicators. The PER (1.87 Vs 1.81) and BV (68 Vs 70%) values of raw and germinated bengal gram were found to be comparable. But, the DC and NPU of germinated bengal gram had significantly improved over the corresponding values of the raw grain. No marked improvement observed in the PER and BV values of the germinated bengal gram protein was attributed to its lower total (6.56 Vs 6.84 g/16g N) and available lysine (75 Vs 85% of the total) contents as compared to those of the raw bengal gram. Jaya and Venkataraman (1980) had also observed

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that supplementation of wheat and rice with germinated bengal gram and green gram respectively did not improve the PER values of the cereal and ungerminated pulse mixture proteins.

Bates et al (1977) had observed that the protein quality of 4 days germinated soya bean in terms of PER did not improve, as a matter of fact, it significantly decreased from that of the raw seeds (from 0.75 to 0.64). But Khader (1983) had observed a 2 fold increase in the PER value from 0.78 to 1.55 of 3 days germinated soya bean protein. Also, Khan and Ghafoor (1978) had exhibited a 2.5 fold increase in the PER value of 2 days germinated mash beans protein. The PER value increased from 0.4 to 1.0 while the NPU (from 44 to 46%), TD (from 72 to 74%) and BV (from 61 to 62%) showed no appreciable improvements.

El-Hag et al (1978) had demonstrated that the DC of the protein of 10 days germinated red kidney bean had increased from 29.5% of the initial value to 66.4%. Earlier in 1973, Palmer et al had observed no change in DC of the kidney bean protein until 4 days of germination but increasing the germination period from 4 to 8 days had resulted in 13% increase in DC values. Likewise, the NPU value increased from a negative value to 3 and 21% after 4 and 8 days of germination respectively. Increases in BV of 48 h germinated bengal gram, black gram and lentil proteins have earlier been demonstrated by Chattopadhyay and Banerjee (1953).

Fotedar (1981) had determined the protein quality of mixes prepared from malted or roasted wheat and bengal gram (4:1) in

terms of PER. The PER value of malted mix protein was significantly higher (2.02 Vs 0.63) than that of the roasted mix protein which was attributed to the increase in available lysine content due to germination.

The protein quality of a mix prepared from malted ragi and green gram (7:3) was determined by NPU, BV and DC as the indicators (Brandtzaeg et al 1981). The total lysine content of malted and unmalted mixes was also determined. The NPU and DC values of the malted and unmalted mixes were comparable while the BV of the malted mix protein was significantly lower than that of the unmalted mix protein which was attributed to the reduction of total lysine content as a consequence of malting (from 368 to 341 and 321 mg/16g N) in 2 varieties of malted ragi and green gram mixes.

Protein quality of baked products

The effects of baking time and temperature on lysine content of bread have been investigated. El-Samahy and Tsen (1981) observed that the lysine content of bread baked at 248°C for 6.4 min was 2.57 g/16g N and it decreased by 19% (from 2.57 to 2.09 g/16g N) when the baking temperature was increased from 248 to 327°C indicating that an increase in baking temperature was deterimental to lysine content. A smaller decrease of 8% was observed when the baking temperature was increased from 327 to 343°C at the baking time of 5.0 min. But no change in lysine content was observed in breads baked at 327 or 343°C for 3.6 or

3.5 min respectively (2.52 Vs 2.53 g/16g N). Also, keeping the baking temperature constant at 327°C, increasing the baking time from 3.6 to 5.0 min did not bring about a change in the lysine content (2.52 Vs 2.50 g/16g N). But, when the baking time was increased from 5.0 to 6.4 min there was a 16% decrease in lysine content. At higher baking temperature (343°C) when the baking time was increased from 3.5 to 5.0 min, a decrease of 7% in the lysine content was observed. These findings suggested that both baking time and temperature exert deterimental effects on the lysine content of the baked product.

In this study, the PER of breads baked at various temperature and for various time periods was also determined. It was observed that the PER values ranged from 1.03 to 1.65 (see below).

Lysine content (g/16g N) of breads baked at different temperature and times; and protein efficiency ratios of bread diets fed to rats for 28 days

	Breads baked at					
	248°C	327°C			343°C	
	6.4 min	3.6 min	5.0 min	6.4 min	3.5 min	5.0 min
Breads: Lysine	2.57	2.52	2.50	2.09	2.53	2.35
Diets: PER	1.65	1.52	1.35	1.03	1.26	1.13

A 38% reduction in the PER values was observed when the baking time was kept constant at 6.4 min and the baking temperature was increased from 248 to 327°C. But, at both the baking time periods of 5.0 and 3.5 min about 17% reduction in PER value was observed with the increase in baking temperature from 327 to 343°C. Increases in baking time also brought about decreases in PER values. At 327 or 343°C when the baking time was increased by 1.4 to 1.5 min, the PER values decreased by 10 to 24%. Hence, the results indicated that both baking time and temperature adversely affect the protein quality of breads (El-Samahy and Tsen 1981).

The effect of heat treatment on available lysine content of casein in presence or absence of carbohydrates has been explored by Smith and Friedman (1984). Casein alone and in combination with carbohydrates such as starch, sucrose or glucose was heated at 37, 121, 200 and 300°C. At 37°C, the ratio of available lysine to total lysine did not appreciably alter in casein heated alone or in the presence of starch or sucrose, while the presence of glucose led to a 35% decrease. But at 121°C, a 15% decrease in available lysine content of casein was observed even in the absence of any carbohydrates whereas in combination with starch, sucrose or glucose the decreases in available lysine content were 20, 66 and 54%, respectively. At 200°C, the pattern of decreases in available lysine content was found to have changed. Here, the greatest decrease occurred in casein alone, 81% as compared to 79% in combination with starch, 57% with sucrose and 48% with glucose. The reason for the protective effect of glucose

on available lysine content at 200°C was attributed to the reduction of heat transfer to the protein caused by partly liquified sugars. The available lysine content of the samples heated at 300°C with the exception of casein alone was zero. These results suggest that heat has a destructive effect on lysine particularly in the presence of carbohydrates.

Clegg (1960) determined the available lysine content of groundnut biscuits containing 8 or 15% skim milk powder (SMP). It was observed that as compared to the lysine content of the unbaked ingredients the available lysine content of the 8% SMP biscuits reduced from 2.82 to 1.79 g/16g N (36%) and that of the 15% SMP biscuits from 2.96 to 2.07 g/16g N (30%).

Prabhavathi et al (1973) evaluated the total lysine content and PER values of baked biscuits against that of unbaked ingredients and commercial biscuit. Three varieties of biscuits were prepared from wheat flour supplemented with protein rich flours such as groundnut, soya bean, wheat germ and pea in the ratio of 20:5:10:0 (biscuit A), 20:25:0:0 (biscuit B) and 20:24:0:0 (biscuit C). It was observed that in comparison to the value of unbaked biscuits baking decreased the PER values by 35, 22 and 13% in biscuits A, B and C, respectively. The PER values of the baked biscuits A, B and C ranged from 1.41 to 2.19 whereas it was 0.98 for the commercial biscuit. It was also observed that the total lysine content of biscuit A decreased from the initial value of 3.93 to 3.60 g/16g N (8%), of biscuit B from 4.13 to

3.81 g/16g N (8%) and of biscuit C from 4.00 to 3.80 g/16g N (5%). The values for available lysine content of biscuits A, B and C were, however, higher than that of the commercial biscuit (2.08 g/16g N).

An year later, Prabhavathi et al (1974) evaluated the protein quality of unbaked mixes and biscuits prepared from these mixes in terms of plasma lysine levels and PER. The biscuits were prepared from wheat flour supplemented with groundnut, soya bean, wheat germ and pea flours in the ratio of 20:5:10:10 (biscuit A) and 20:25:0:0 (biscuit B). The weanling rats were fed diets containing baked (biscuit) or unbaked mixes (controls). As compared to the plasma lysine levels of the controls, those of biscuit A fed rats were lower by 69% (from 26.8 to 8.3 mcg/ml plasma) and of biscuit B fed rats by 67% (from 28.5 to 9.4 mcg/ml plasma). Likewise, the PER values of biscuits A and B diets were 35% (2.18 Vs 1.41) and 22% (1.99 Vs 1.55) respectively lower than those of the control values. These findings indicated that baking decreased the protein quality of biscuits.

Recently, Bjorck et al (1983) determined the effects of extrusion cooking on the available lysine content of biscuits. The biscuits were baked at 170, 193 and 210°C. In order to see the effect of moisture content on the retention of total and available lysine, the biscuits were baked with 13 or 18% moisture at 210°C. It was observed that as compared to the values of the raw material there were 14, 22 and 38% decreases in total lysine

contents of biscuits baked at 170, 193 and 210°C, respectively. Likewise, decreases in the available lysine contents were also observed. The decreases in available lysine in comparison to the values of the raw material were 7% in biscuits baked at 170°C and 16 and 36% in biscuits baked at 193 and 210°C, respectively (see below). At 210°C, the retention in total and available

Total and available lysine contents of raw material and extruded biscuits

	g/16g N				
		Biscuits baked at			
	Raw material	170°C	193°C	210°C	210°C
		moisture contents (%) of the feed			
		13	13	13	18
Total lysine	6.3	5.4	4.9	3.9	4.5
Available lysine	5.8	5.4	4.9	3.7	3.9

lysine contents were 15 and 5% higher in 18% moisture biscuits as compared to the 13% moisture biscuits indicating that the moisture content of biscuit exerted a beneficial effect on lysine retention.

Hernandez and Sotelo (1984) determined the protein quality of cookies. The authors prepared cookies from a mixture of wheat

and bengal gram flours in the ratio of 60:40 (by weight). The mixture and the cookies were analysed for available lysine content. PER determinations were also made. It was observed that the available lysine content of the mixture was higher than that of the wheat flour alone (4.39 Vs 2.00 g/16g N) probably because of the higher available lysine content of the bengal gram flour (6.35 g/16g N) as compared to that of the wheat flour (2.00 g/16g N). However, the available lysine content of the cookies was lower than that of the mixture (3.46 Vs 4.39 g/16g N) which was attributed to the reduction of available lysine due to baking. The PER value of the mixture protein (2.31) was higher than that of wheat (0.63), and bengal gram (2.11) proteins alone, and of the cookie protein (1.82).

This experiment was planned to evaluate the growth promoting ability and protein quality of mixes and biscuits by the methods based on growth rate such as PER, by determining the availability of lysine, by those based on nitrogen balance such as NPU, BV and DC, by those based on tissue protein levels such as hepatic protein content and serum protein and serum urea concentrations.

Materials and methods

As described in Chapter 3, wheat and bengal gram grains in the ratio of 4:1 by weight, were soaked for 12 h and germinated for 48 h 22°C (12 to 34°C). The germinated grains with rootlets were dried in an oven at 70±5°C for 9 to 11 h and milled. Mixes

made from malted and raw wheat and bengal gram were used in the preparation of biscuits. Biscuits were prepared from 40 g of mix, 40 g of jaggery and 20 g of vanaspati with or without 7.5 g of colocasia leaf powder.

Animal experiment

Experimental diets : The composition of the experimental diets is presented in Tables 58 and 59. The basal diet contained casein as the protein source and the experimental diets contained malted and raw mixes, and biscuits made from these mixes with or without colocasia leaf powder as the protein sources. The water soluble vitamin mixture (Table 60) and the mineral mixture (Table 61) were those of Rajalakshmi et al (1969) and Oser (1979), respectively. The fat soluble vitamins were added to the diet as recommended by National Academy of Sciences (1978).

Experimental plan : For PER experiment, one group of 24 weanling rats weighing between 35 to 50 g were divided into 3 groups of 8 rats each. They were fed for 28 days, diets containing casein or malted mix or raw mix to provide 10% protein (Table 58). Since biscuits contained 6% protein another group of 56 weanling rats weighing between 35 to 50 g was divided into 7 groups of 8 rats each, and were fed for 28 days diets containing casein, malted and raw mixes, and biscuits prepared from malted and raw mixes with or without colocasia leaf powder providing 6% protein (Fig 12). Records on food intake and weight change were maintained

Table 58. Composition of the diets used for the PER experiments

Ingredients (g/100 g)	Experimental diets									
	Malted mix	Raw mix	Casein	MM biscuits	RM biscuits	C-MM biscuits	C-RM biscuits	Malted mix	Raw mix	Casein
Malted mix	80.2	-	-	-	-	-	-	48.1	-	-
Raw mix	-	82.6	-	-	-	-	-	-	49.6	-
Casein[a]	-	-	11.8	-	-	-	-	-	-	7.1
MM biscuits	-	-	-	97.0	-	-	-	-	-	-
RM biscuits	-	-	-	-	97.0	-	-	-	-	-
C-MM biscuits	-	-	-	-	-	97.0	-	-	-	-
C-RM biscuits	-	-	-	-	-	-	97.0	-	-	-
Groundnut oil[b]	8.2	8.1	10.0	-	-	-	-	21.0	21.0	21.0
Vitamin mix[c]	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0
Mineral mix[d]	2.0	2.0	4.0	2.0	2.0	2.0	2.0	2.0	2.0	4.0
Corn starch[b]	8.6	6.3	72.2	-	-	-	-	27.9	26.4	65.9
Protein[e] (g)	9.86	9.88	9.74	6.52	6.33	7.23	7.03	5.94	5.91	5.90
Calories[f] (Kcal)	388	386	426	493	493	490	490	468	468	481

[a] Amul Dairy, Anand, protein 85%
[b] Local market
[c] Rajalakshmi et al (1969)
[d] Oser (1979)
[e] Analysed values
[f] Calculated (Gopalan et al 1985)

Table 59. Composition of diets used for the NPU, BV and DC experiments

Ingredients (g/100 g)	Experimental diets								
	Protein free	Malted mix	Raw mix	Casein	MM biscuits	RM biscuits	C-MM biscuits	C-RM biscuits	Casein
Malted mix	-	76.2	-	-	-	-	-	-	-
Raw mix	-	-	77.5	-	-	-	-	-	-
Casein[a]	-	-	-	11.8	-	-	-	-	7.1
MM-biscuits	-	-	-	-	97.0	-	-	-	-
RM biscuits	-	-	-	-	-	97.0	-	-	-
C-MM biscuits	-	-	-	-	-	-	97.0	-	-
C-RM biscuits	-	-	-	-	-	-	-	97.0	-
Groundnut oil[b]	10.0	8.3	8.2	10.0	-	-	-	-	10.0
Vitamin mix[c]	2.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	2.0
Mineral mix[d]	4.0	2.0	2.0	4.0	2.0	2.0	2.0	2.0	4.0
Corn starch[b]	84.0	12.5	11.3	72.2	-	-	-	-	76.9
Protein[e] (g)	-	10.16	10.16	9.97	6.40	6.18	7.00	6.87	5.95
Calories[f] (Kcal)	426	391	386	426	493	493	490	490	426

See Table 58 for foot note.

Table 60. Composition of water soluble vitamin mixture[a]

Vitamins	Amount
Thiamine hydrochloride (mg)	1.5
Riboflavin (mg)	2.5
Pyridoxine hydrochloride (mg)	1.0
Niacine (mg)	5.0
Calcium-d-pantothenate (mg)	10.0
Choline chloride (mg)	500.0
Inositol (mg)	200.0
Para amino benzoic acid (mg)	10.0
Folic acid (mg)	1.0
Cyanocobalamine (mcg)	5.0
Biotin (mcg)	1.0
Powdered sugar	to make a total weight of 20 g

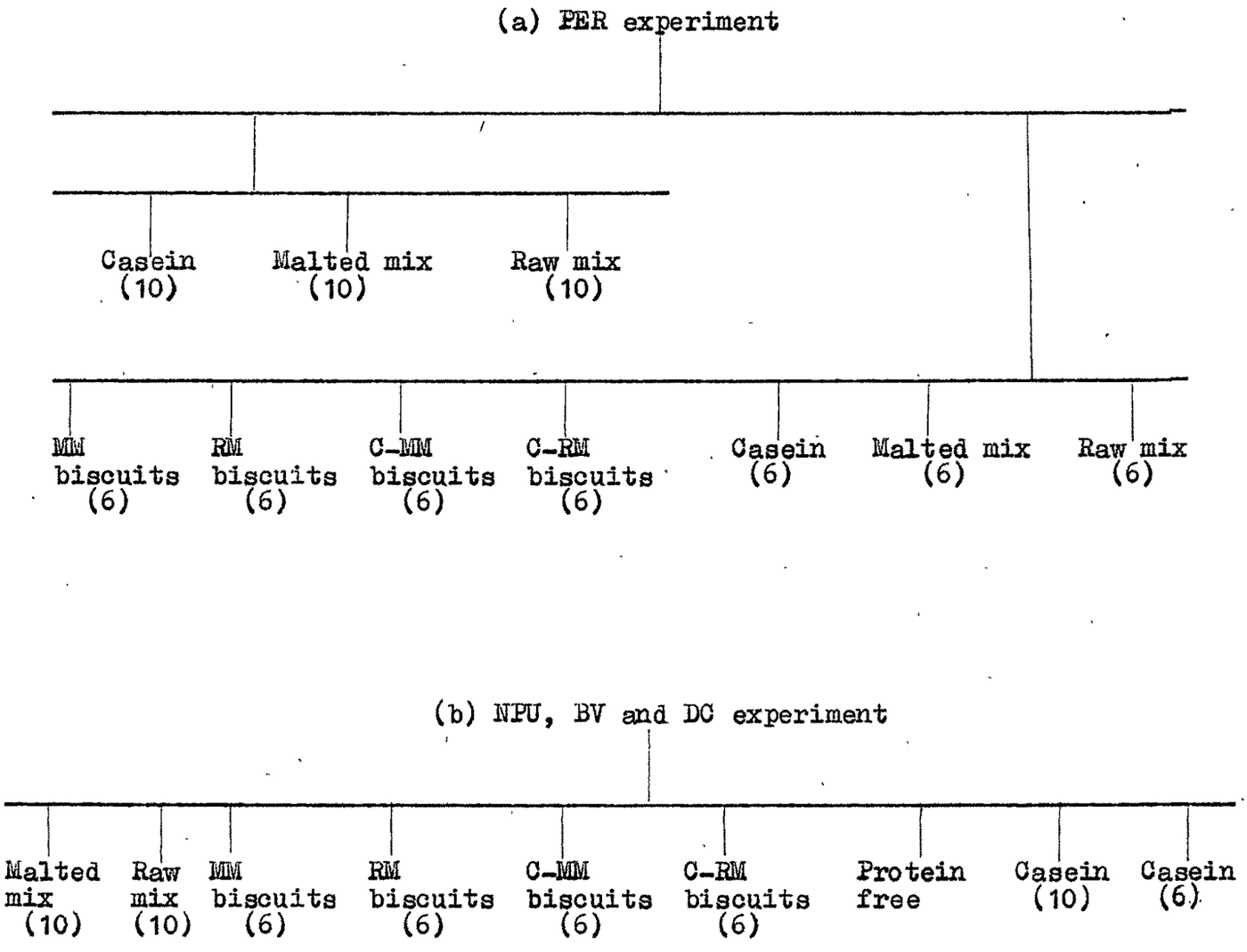
[a]To be used at 2% level in the diet

Table 61. Composition of the Hawk-Oser salt mixture No. 4*

Mineral salts		Amounts (g)
Ca Citrate.$4H_2O$		308.2
$Ca(H_2Po_4)_2.H_2O$		112.8
K_2HPo_4		218.7
KCl		124.7
NaCl		77.0
$CaCO_3$		68.5
$3MgCo_3.Mg(OH)_2.3H_2O$		35.1
$MgSo_4$(anhydrous)		38.3
$FeNH_4$ citrate	91.36	
$CuSo_4.5H_2O$	5.97	
NaF	0.76	
$MnSo_4.2H_2O$	1.07	16.7
$KAl(So_4)_2.12H_2O$	0.54	
KI	0.24	
$ZuSo_4.H_2O$	0.06	
	100.00	1000.0

*To be used at 4% level in the diet

Fig 12. Experimental plan



Figures in parenthesis denote the level of protein in the diet

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and PER values were calculated (Evans and Witty 1978).

For NPU, BV and DC determinations, 72 rats weighing between 55 to 65 g were divided into 9 groups of 8 each (Fig 12). One group was placed on protein free diet and the remaining groups on experimental diets (Table 59) for a period of 10 days. During the last 3 days of the experimental period, the rats were placed in metabolic cages for urine and feces collection. The urine was collected under toluene. The fecal pallets were collected, air and oven dried and stored until analysed. The diets were fed ad libitum. The diets, urine and fecal samples were analysed for nitrogen by micro-Kjeldahl procedure (Ranganna 1977). NPU, BV and DC of the proteins were calculated (Evans and Witty 1978).

Calculations:

$$PER = \frac{\text{Weight gain (g)}}{\text{total protein intake (g)}}$$

$$NPU = \frac{NI - (FN-MFN) - (UN-EUN)}{NI} \times 100$$

$$BV = \frac{NI - (FN-MFN) - (UN-EUN)}{NI - (FN-MFN)} \times 100$$

$$DC = \frac{NI - (FN-MFN)}{NI} \times 100$$

where : NI = nitrogen intake (mg)
FN = fecal nitrogen (mg)
MFN = metabolic fecal nitrogen (mg)
UN = urine nitrogen (mg)
EUN = endogenous urinary nitrogen (mg)

Autopsy procedure for PER experiment : At the time of the autopsy the rats were weighed and ether anaesthetised. The liver was

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removed and freed from extra hepatic tissues and blotted on damp filter paper, weighed and sampled for hepatic protein estimation. The samples and the remaining tissues were wrapped separately in aluminium foil and kept in the deep freeze until analysed.

Analytical procedure

Nitrogen content: The mixes, biscuits, and diets, urine and feced were analysed for their nitrogen contents. The procedure followed was the same as described in Chapter 3.

Available lysine content: Available lysine was estimated by the method of Carpenter (1960). The lysine residues with reactive epsinol-NH_2 groups in the food proteins are converted into the yellow epsinol-DNP-lysine by treatment of the material with fluoro-2:4-dinitrobenzene (FDNB) followed by acid hydrolysis. Ether soluble interfering compounds are removed by extraction and the extinction of the residual acqueous layer is measured. A blank value is obtained by treatment with methoxy-carbonyl chloride and extraction of the ether soluble lysine compound which results. Reagents used for the estimation of available lysine are given in Appendix 11.

In stage 1, finely ground samples, in duplicate, each containing an estimated 30 to 50 mg of nitrogen, were taken into round bottomed flasks and to each was added 8 ml of 8% (w/v) sodium bicarbonate. They were shaken gently to disperse the material and then left for 10 min. FDNB (0.3 ml) previously

dissolved in 12 ml of ethanol, was added to each flask, which was then stoppered and shaken gently on a mechanical shaker for 2 h. The stoppers were removed and the flasks stood in boiling water until there was no more effervescence even on shaking. Then 8.1 N hydrochloric acid (24 ml) was added immediately and the flasks were refluxed gently for 16 h with condensers adequate to prevent loss of hydrochloric acid. The flasks were then disconnected after washing the condensers with water. After the flasks had stood in ice water for one to 2 h, the contents were filtered through a Whatman No. 41 filter paper with water washings and the filtrate was made up to 250 ml.

In stage 2, 2 ml portions from each filtrate were pipetted into 2 glass stoppered tubes marked A and B, graduated at 10 ml, and into a small conical flask. The contents of the tubes were extracted twice with 5 ml (approximate) portions of ether, the ether layers were discarded and the tubes were held in boiling water until effervescence from the residual ether ceased, and the tubes were cooled. The volume of tube A was made up to 10 ml with N hydrochloric acid and kept aside for the final readings.

In stage 3, the contents of flask C were titrated with 10% (w/v) sodium hydroxide with phenolphthelein as the indicator to the end point of pink colour. The volume of sodium hydroxide taken by the content of conical flask was then added to tube B, followed by 2 ml of buffer solution, pH 8.5. Methoxy-carbonyl chloride (0.045 to 0.055 ml) was then added and the tube shaken vigorously to disperse and dissolve the compound. After 5 to 10 min,

0.75 ml of concentrated hydrochloric acid was added, cautiously at first and with agitation to prevent the contents frothing over. The contents were again extracted twice with 5 ml of ether. The residual ether in the aqueous layer was evaporated by standing the tubes in boiling water, and the volume was made to 10 ml with water.

In stage 4, the extinction coefficients of the contents of tubes A and B were measured at 435 nm in Spectronic 20. 'Reading A - Reading B' was compared with the corresponding values obtained with 2 ml of standard solutions containing 30 to 60 mcg DNP-lysine passed through the procedure from stage 2 onwards, with omission only of the ether washings in stage 2.

Calculation:

$$\text{Available lysine (g/16g N)} = \frac{SRA-SRB}{SRA_1-SRB_1} \times C \times \frac{V}{V_1} \times \frac{100\ g}{S} \times \frac{100\ g}{P} \times 0.945 \times 0.468 \times \frac{1}{10^6}$$

where:

SRA	=	reading A of the sample
SRB	=	reading B of the sample
SRA_1	=	reading A of the standard
SRB_1	=	reading B of the standard
C	=	concentration of the standard (mcg)
V	=	total volume after acid hydrolysis (ml)
V_1	=	aliquot taken for estimation (ml)
S	=	weight of the sample taken for analysis (g)
P	=	protein in 100 g of sample (g)
0.945	=	conversion of epsilon-DNP-lysine HCl to epsilon-DNP-lysine
0.468	=	conversion of epsilon-DNP-lysine to lysine.

Hepatic protein content: Hepatic protein was determined by the method of Lowry et al (1951) with the modification suggested by Munro and Fleck (1969) for the preparation of reagent B. In the procedure for the estimation of hepatic protein, there are 2 distinct steps which lead to the final colour development with protein; reaction with copper in alkali and the reduction of the phosphomolybdic phosphotungstic reagent by the copper treated protein. The reagents used for the estimation of hepatic protein are given in Appendix 12.

About 200 mg of the sampled liver tissue was homogenised in one millilitre of phosphate buffer, pH 7.0. The volume was made up to 3.0 ml with the buffer in a centrifuge tube. An aliquot of 0.5 ml homogenate was transferred into a test tube containing 0.5 ml of 10% trichloroacetic acid (TCA) to precipitate the protein. The tubes were placed in an ice bath for 10 min and the contents centrifuged at 2500 rpm for 10 min. The clear supernatant was discarded. The process was repeated once again using 5% TCA. The precipitate was then dissolved in one millilitre of N sodium hydroxide. The tubes were kept in a boiling water bath for 5 min. The contents were cooled to room temperature and the volume was made up to 10 ml with distilled water. The tubes were kept in a refrigerator until analysed.

Aliquots of 0.1 ml of the extract were taken in duplicates. The volume was made up to one millilitre with 0.1 N sodium hydroxide. Five millilitres of alkaline copper reagent was added and the contents mixed. After 10 min, 0.5 ml of diluted folin

ciocalteau reagent was added and the contents were shaken immediately. A blank, and standard solutions containing 20 to 100 mcg of bovine serum albumin were run with each set of estimations. The colour was read in the Klett Summerson colorimeter at 660 nm after 30 min.

Calculation:

$$\text{Hepatic protein (g/100 g liver)} = \frac{SR}{SR_1} \times C \times \frac{VE}{V_1} \times \frac{V}{VE_1} \times \frac{100\ g}{S} \times \frac{1}{1000}$$

where: SR = reading of the sample
SR_1 = reading of the standard
C = concentration of the standard (mg)
V = volume after buffer addition (ml)
VE_1 = aliquot taken for precipitation (ml)
VE = volume made up after precipitation (ml)
V_1 = aliquot taken for estimation (ml)
S = weight of liver taken for analysis (g)

Serum protein content: Serum protein was estimated using birut method as modified by Varley et al (1980). In the biuret reaction, substances which contain two-$CO.NH_2$ groups joined together directly or through a single carbon or nitrogen atom, and those which contain 2 or more peptide links, give a blue to purple coloured compound with alkaline copper solutions. One copper atom complexes with 4 molecules of biuret, the linkages being to the central nitrogen atom. The reagents used for the estimation of serum proteins are given in Appendix 13.

In a test tube, 6.0 ml of sulphate-sulphite solution was taken and 0.4 ml of serum was added. The contents were mixed by inversion. Two millilitres of the above mixture was added to 5.0 ml of biuret reagent. Serum blank was prepared by the addition of 2.0 ml of serum-sulphate-sulphite mixture to 5.0 ml tartrate-iodide solution. The biuret blank contained 2.0 ml of sulphate-sulphite solution in 5.0 ml of biuret reagent. Standard solutions containing 1.6 to 8.0 mg/ml of bovine serum albumin were treated in a similar manner as the sample.

All the tubes were placed in the water bath at 37°C for 10 min. The tubes were cooled for 5 min at room temperature and the colour intensity was read at 540 nm in the Klett Summerson colorimeter. The serum blanks were read against the tartrate-iodide solution and the tests and standards against the biuret blank.

Calculation:

$$\text{Serum protein (g/dl)} = \frac{SR}{SR_1} \times C \times \frac{V}{V_1} \times \frac{100 \text{ ml}}{S} \times \frac{1}{1000}$$

where: SR = reading of the sample
SR_1 = reading of the standard
C = concentration of the standard (mg)
V = total volume after sulphate-sulphite addition (ml)
V_1 = aliquot taken for estimation (ml)
S = serum taken for analysis (ml)

Serum urea content: Serum urea was determined by diacetyl monoxime method as described in Varley et al (1980). When urea is heated

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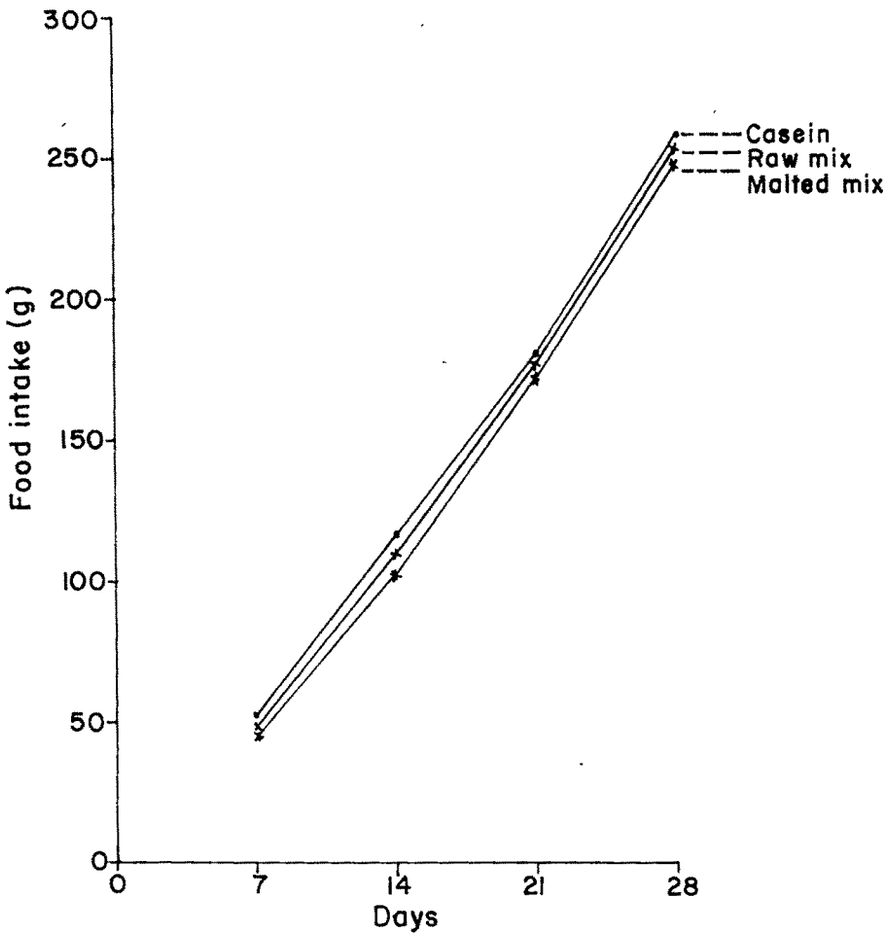
Fig 13. Weekly changes in food intake of malted mix, raw mix and casein diets fed rats (10 % protein diets)



the weight gain of the malted or raw mix diet fed rats was lower than that of those fed casein diet (Fig 14). After 7 days of the experiment the growth rate in terms of weight gain of mix diets fed rats was 30% less than that of casein diet fed rats. This difference in mean body weights between mixes and casein diets fed rats widened as the experiment progressed from 7 to 28 days. It was of 7 g on the seventh day, of 19 g on the fourteenth day, of 28 g on the twentyfirst day and of 42 g on the twentyeighth day of the experiment. The progressive retardation of growth in rats fed cereal-pulse diet was attributed to the intake of protein of low biological value. Between the 2 mixes fed groups, the weight gain at each weekly interval did not vary from each other.

Table 63 exhibits that malting of wheat and bengal gram did not markedly improve the quality of mix protein in terms of its available lysine content. The available lysine content of malted mix was only 5% higher than that of the raw mix (3.61 Vs 3.42 g/16g N). The increase in available lysine content of germinated wheat (Hamad and Fields 1979) perhaps was counteracted by the decrease in that of germinated bengal gram (Geervani and Theophilus 1980, Jaya and Venkataraman 1980), consequently the net gain in available lysine was found to be negligible.

In the present study, the available lysine content of the mix prepared from raw wheat and bengal gram in the ratio of 4:1 by weight was 3.42 g/16g N (Table 63). Earlier, Hernandez and Sotelo (1984) had reported that the available lysine content of mix prepared from raw wheat and bengal gram in the ratio of 6:4

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Fig 14. Weekly changes in weight gain of the malted mix, raw mix and casein diets fed rats (10 % protein diets)

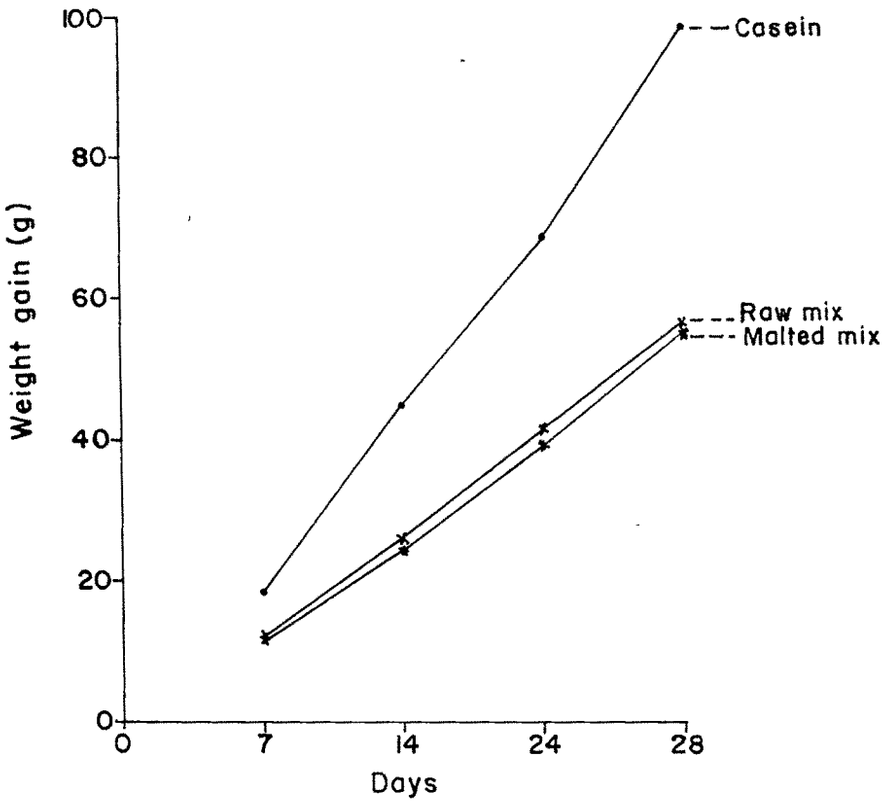

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Table 63. Protein quality of malted mix, raw mix and casein diets

Groups	Available lysine g/16g N	PER	NPU %	BV %	DC %
			Mean ± SE		
Malted mix	3.61 ±0.420	2.28 ±0.059	73.0 ±3.059	85.0 ±2.538	85.7 ±1.115
Raw mix	3.42 ±0.425	2.25 ±0.094	61.6 ±2.368	72.6 ±2.054	84.8 ±1.562
Casein	-	3.90 ±0.096	72.1 ±6.257	80.1 ±5.079	95.5 ±0.792

't' values for various comparisons

Comparisons	't' values				
Malted Vs raw mix	0.318 NS	0.270 NS	2.947*	3.798**	0.469 NS
Malted mix Vs casein	-	14.336***	0.129 NS	0.863NS	7.169***
Raw mix Vs casein	-	12.313***	1.570 NS	1.369NS	6.111***

NS Non Significant

* Difference between means significant at 5% level of significance

** Difference between means significant at 1% level of significance

*** Difference between means significant at 0.01% level of significance

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by weight was 4.39 g/16g N. The relatively higher available lysine content of the latter than the former mix (4.39 Vs 3.42 g/16g N) was due to the higher amount of bengal gram in relation to wheat (4:2.7 Vs 4:1) in the latter than the former mix as the available lysine content of bengal gram is higher than that of wheat (Hernandez and Sotelo 1984). Since the values for available lysine contents of wheat as 2.00 (Hernandez and Sotelo 1984), 2.07 (Chawla and Kapoor 1982) and 2.89 g/16g N (Reddy 1971) and of bengal gram as 4.49 (Jaya and Venkataraman 1980), 6.35 (Hernandez and Sotelo 1984) and 6.39 g/16g N (Murthy and Urs 1980) were available, it was considered worthwhile to calculate using these values, the available lysine content of wheat and bengal gram mixed in the ratio of 4:1. It was calculated to be 3.39 g/16g N. This value was found to be quite comparable to the value observed in the present study (3.39 Vs 3.42 g/16g N).

The mean PER values (Table 63) of the malted (2.28) and raw (2.25) mix proteins did not differ from each other but these values were significantly lower than that of the casein (3.90) protein. The PER value of 2.28 for the malted mix protein observed in the present study compared well with that of 2.02 reported by Fotedar (1981) for similar malted wheat bengal gram mix protein. No difference in the mean PER values of malted and raw mix proteins was perhaps due to the fact that there was no appreciable difference in the available lysine contents of the malted and raw mixes (3.61 Vs 3.42 g/16g N). These data also indicated that when the ratio of wheat to bengal gram protein

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was 1:0.36, the PER value of the mix protein was 2.25. This value was quite comparable with that reported by Phansalkar et al (1957) for a mix prepared from wheat and bengal gram in the protein ratio of 1:0.43. Also, the PER of a wheat and bengal gram mix (Hernandez and Sotelo 1984) prepared in the ratio of 6:4 by weight to provide equal amounts of protein (1:1.1) did not markedly vary from that observed in the present study (2.31 Vs 2.25). Thereby indicating that an increase in the ratio of the bengal gram protein in a wheat bengal gram mix did not appreciably improve the PER values. In contrast, Chandrasekharappa (1979) had reported that the PER value of mix containing 88% wheat and 12% bengal gram having cereal to legume protein ratio of 1:0.25 tended to be lower than that of a mix containing 73% wheat and 27% bengal gram with protein ratio of 1:0.67 (2.09 Vs 2.48). The PER value of 2.25 observed in the present study of a mix having cereal to legume protein ratio of 1:0.36 was comparable with that of 2.48 reported by Chandrasekharappa (1979) for the mix containing cereal legume protein ratio of 1:0.67. Del-angel and Sotelo (1982) have reported that when the bengal gram protein proportion was increased from 0.43 to 0.67 to 1.0 in a wheat bengal gram mix, the PER values did not change appreciably.

The finding that malting did not markedly improve the protein quality in terms of PER was in accordance with the results of Jaya and Venkataraman (1979), Hemanalini et al (1980) and Geervani and Theophilus (1980) but differed from those of Bates et al (1977) and Khan and Ghafoor (1978). Hemanalini et al (1980) had observed

no improvement in the PER value of germinated ragi. Earlier Jaya and Venkataraman (1979) had made similar observations in germinated bengal gram and green gram. Likewise, Geervani and Theophilus (1980) had reported no difference in PER values between the raw and 16 h germinated bengal gram (1.87 Vs 1.81). Bates et al (1977) had reported a significant decrease in the PER value from 0.75 to 0.64 of 0 to 96 h germinated soya beans. On the other hand, Khan and Ghafoor (1978) had exhibited a 2.5 fold increase (from 0.4 to 1.0) in the PER value of 48 h germinated mash beans. The authors attributed the improvement in protein quality of mash beans to the hydrolysis of protein resulting in the better digestibility of the proteins.

Unlike the PER values, the mean NPU and BV values of the malted mix protein were significantly higher (Table 63) than those of the raw mix protein while the mean value for DC although tended to be higher did not significantly differ from that of the raw mix protein. It seems from these data that the procedure of malting improved the protein quality of wheat and bengal gram but the DC of protein was not markedly improved.

Earlier, somewhat lower NPU (57.3 Vs 61.3%) and BV (65.1 Vs 71.0%) values and somewhat higher DC (88.1 Vs 86.3%) values for protein of the malted ragi and green gram (7:3) mix in comparison to those of unmalted mix were reported by Brandtzaeg et al (1981). While in the present study, significantly higher NPU (73.0 Vs 61.6%) and BV (85.0 Vs 72.6%) and comparable DC (85.7 Vs 84.8%) values for the protein of the malted mix were observed in

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comparison to those of the raw mix protein. The discrepancy in results of the present study and those of Brandtzaeg et al (1981) might be due to the differences in the type of cereal-pulse (wheat and bengal gram Vs ragi and green gram) and the ratio in which they were used (4:1 Vs 7:3). Moreover, there were variations in germination periods, in the present study the grains were germinated for 48 h while Brandtzaeg et al (1981) had germinated ragi for 30 h and green gram for 24 h.

A few investigators have reported improvement in protein quality of legumes as a consequence of germination. Geervani and Theophilus (1980) have reported that 16 h germinated bengal gram had somewhat higher NPU (79 Vs 73%) and DC (55 Vs 49%) and comparable BV (70 Vs 68%) values to those of the unprocessed bengal gram. El-Hag et al (1978) had earlier observed an increase in the DC value from 30 to 66% of kidney beans germinated for 10 days. Palmer et al (1973) had demonstrated that NPU increased from a negative value of ungerminated kidney beans to 3 and 21% in beans germinated for 4 and 8 days, respectively. The authors observed no change in the true digestibility value of 4 days germinated beans in comparison to that of the ungerminated beans but it increased from 55 to 62% after 8 days of germination. On the other hand, Khan and Ghafoor (1978) had demonstrated negligible improvement in the protein quality of 48 h germinated mash beans, the values for NPU were 44 and 46%; TD, 72 and 74%; and BV, 61 and 62% for the raw and 48 h germinated mash beans, respectively.

The comparison between malted mix and casein protein indicated that the mean values for NPU and BV of the malted mix and those of casein protein were comparable (Table 63). The mean DC value of the former protein, however, was significantly lower (85.7 Vs 95.5%) than that of the latter protein. A similar reduction in DC of the malted ragi and green (7:3) mix in comparison to the casein protein (88.1 Vs 99.5%) has earlier been reported by Brandtzaeg et al (1981). Similar observations were made in raw mix protein. The mean values for NPU and BV of the raw mix protein were comparable to that of casein protein but that of DC was lower (Table 63).

The results of the evaluation of protein quality by tissue protein levels of the malted and raw mixes and casein diet fed rats are presented in Table 64. The mean hepatic protein content of the rats fed malted mix diet was significantly lower than those of the rats fed raw mix and casein diets. But no significant difference was observed between the mean hepatic protein contents of casein and raw mix diet fed rats.

The mean serum protein concentrations of the rats fed malted and raw mix diets did not differ from each other but were significantly lower than that of the casein fed rats (Table 64). These findings are in accordance with those of Fotedar (1981) who had reported that serum protein concentration of the malted mix diet fed rats were significantly lower than that of the casein diet fed rats.

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Table 64. Hepatic protein, serum protein and serum urea contents of rats fed malted mix, raw mix and casein diets

Groups	Hepatic protein g/100 g	Serum protein g/dl	Serum urea mg/dl
		Mean ± SE	
Malted mix	14.2±0.435	5.26±0.178	28.24±1.954
Raw mix	15.8±0.536	5.59±0.161	31.26±3.102
Casein	16.2±0.549	6.61±0.150	20.03±1.726

't' values for various comparisons

Comparisons	't' values		
Malted Vs raw mix	2.319*	1.375 NS	0.824 NS
Malted mix Vs casein	2.857**	5.794***	3.149**
Raw mix Vs casein	0.522 NS	4.636***	3.163**

See Table 63 for foot note

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The concentration of urea in serum reflects the quality of ingested protein. Gross et al (1982) have demonstrated that poor quality protein and low protein content in the diets increases the blood urea concentration. Schoenberger and Gross (1982) have opined that the intake of an unbalanced protein results in an increase in the blood urea concentration.

In the present study, the mean serum urea concentrations of the malted mix diet fed rats exhibited a tendency to be lower than that of the raw mix diet fed rats although the values did not significantly differ from each other (Table 64). Between the mixes and casein diets fed rats the mean serum urea levels of the former rats were significantly higher than that of the latter rats (Table 64). These findings were attributed to the inferior protein quality of vegetable proteins. In casein diet fed rats the mean value for serum urea concentration observed in the present study (20.03 mg/dl) was comparable with the value of 20.3 mg/dl reported by Gross et al (1982) and was somewhat lower than the value of 25.5 mg/dl reported by Schoeneberger and Gross (1982).

Somewhat lower serum urea concentration in malted versus raw mix diet fed rats was ascribed to the higher mean NPU, BV and DC values of the malted mix protein than those of the raw mix protein. These data did give an indication that malting of wheat and bengal gram tended to improve the protein quality of these grains.

Growth promoting ability and protein quality of biscuits

In the entire 28 days experimental period the MM biscuit diet fed rats lost about 6 g of weight while those fed RM biscuit diet lost one gram of weight (Table 65). The difference in mean body weights between the 2 groups was statistically significant (Table 66). Since the mean food intake of rats fed MM or RM biscuit diet was comparable, it indicated that the former diet was not utilized for growth as efficiently as was the latter diet. Figure 15 shows that the loss in weight of rats fed MM biscuit diet increased as the experiment progressed. It was of 2.0 g on the seventh day and 5.6 g on the twentyeighth day. On the other hand, the rats fed RM biscuit diet lost weight during the first 7 day period but thereafter exhibited an improvement in growth as the mean weight loss was 2.1 g on the seventh day and one gram on the twentyeighth day. Since the rats fed MM biscuit diet lost relatively more weight it therefore follows that from the nutritive point of view, malted mix should not be subjected to further heat treatment at a temperature as high as that required in preparation of biscuits.

Earlier, Ranhotra et al (1977) had prepared bread from 3 to 5 days germinated wheat flour and had also observed no improvement in the PER value. The authors attributed the absence of improvement in the protein quality of bread to the loss of available lysine during heat treatment of germinated wheat.

Comparing the growth rate of rats fed diets containing biscuits and mixes it was noted that the rats fed biscuit diets

Table 65. Weight change and food intake of rats fed biscuits, mixes and casein diets (28 days)

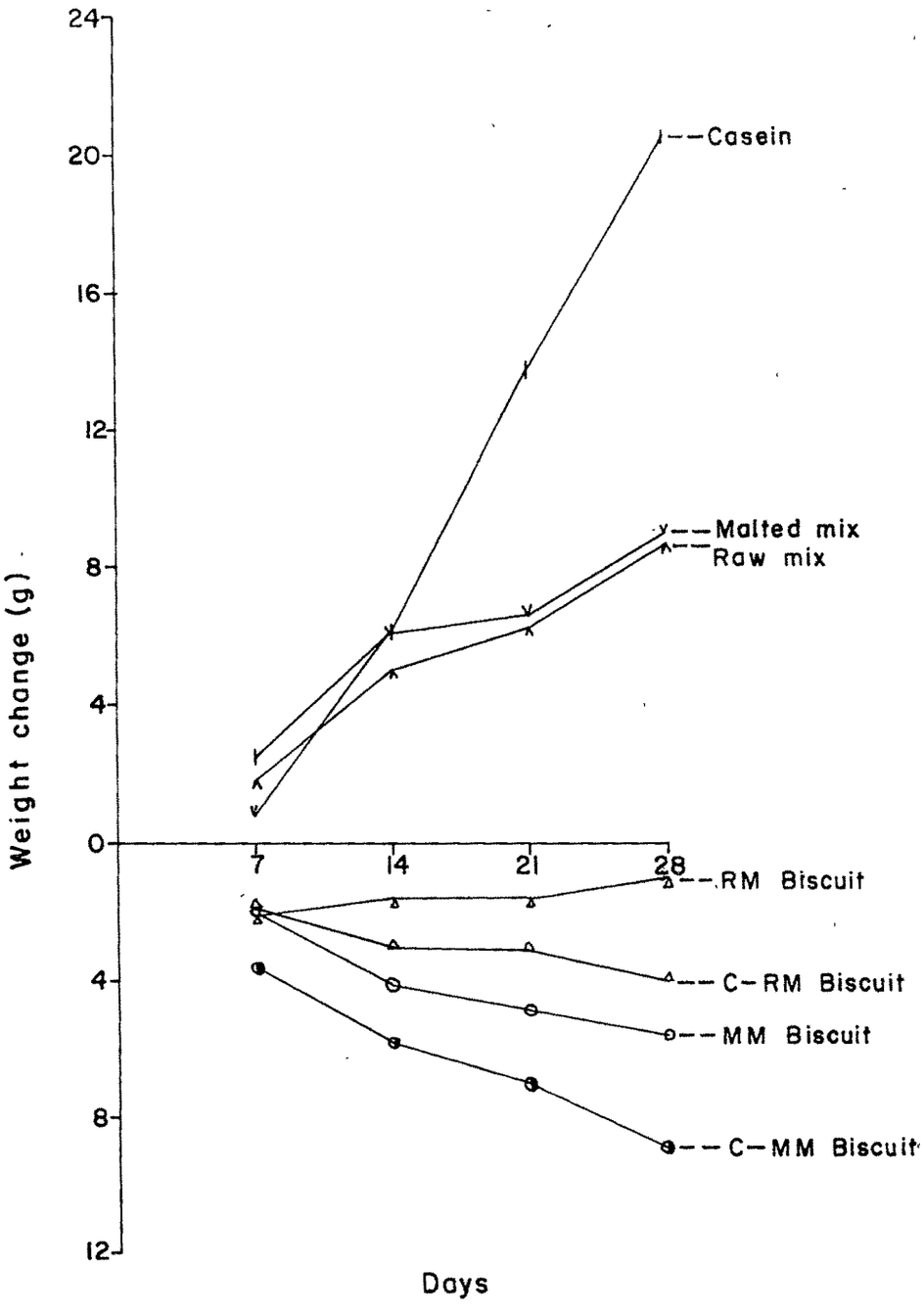
Groups	Weight change g	Food intake g
	Mean ± SE	
MM biscuits	-5.6±0.804	90.8±3.009
RM biscuits	-1.0±0.840	98.5±7.766
C-MM biscuits	-8.9±1.302	82.1±3.262
C-RM biscuits	-4.0±0.968	93.9±4.184
Malted mix	+9.0±0.962	124.6±12.576
Raw mix	+8.7±2.130	121.2±8.562
Casein	+20.6±1.790	131.9±5.212

Table 66. 't' values for the variables of Table 65

Comparisons	Weight change	Food intake
Among biscuits		
MM Vs RM biscuit	3.955**	0.942 NS
MM Vs C-MM biscuit	2.157*	1.960 NS
RM Vs C-RM biscuit	2.340*	0.522 NS
C-MM Vs C-RM biscuit	3.021**	2.224*
Between biscuits and mixes		
MM biscuit Vs malted mix	11.643***	2.614*
RM biscuit Vs raw mix	4.236***	1.964 NS
C-MM biscuit Vs malted mix	11.056***	3.271**
C-RM biscuit Vs raw mix	5.427***	2.865*
Between biscuits and casein		
Malted mix Vs casein	5.709***	0.536 NS
Raw mix Vs casein	4.277***	1.067 NS
MM biscuit Vs casein	13.354***	6.830***
RM biscuit Vs casein	10.926***	3.571**
C-MM biscuit Vs casein	13.330***	8.099***
C-RM biscuit Vs casein	12.088***	5.685***

See Table 63 for foot note

Fig 15. Weekly changes in weights of the biscuits, mixes and casein diets fed rats (6 % protein diets)



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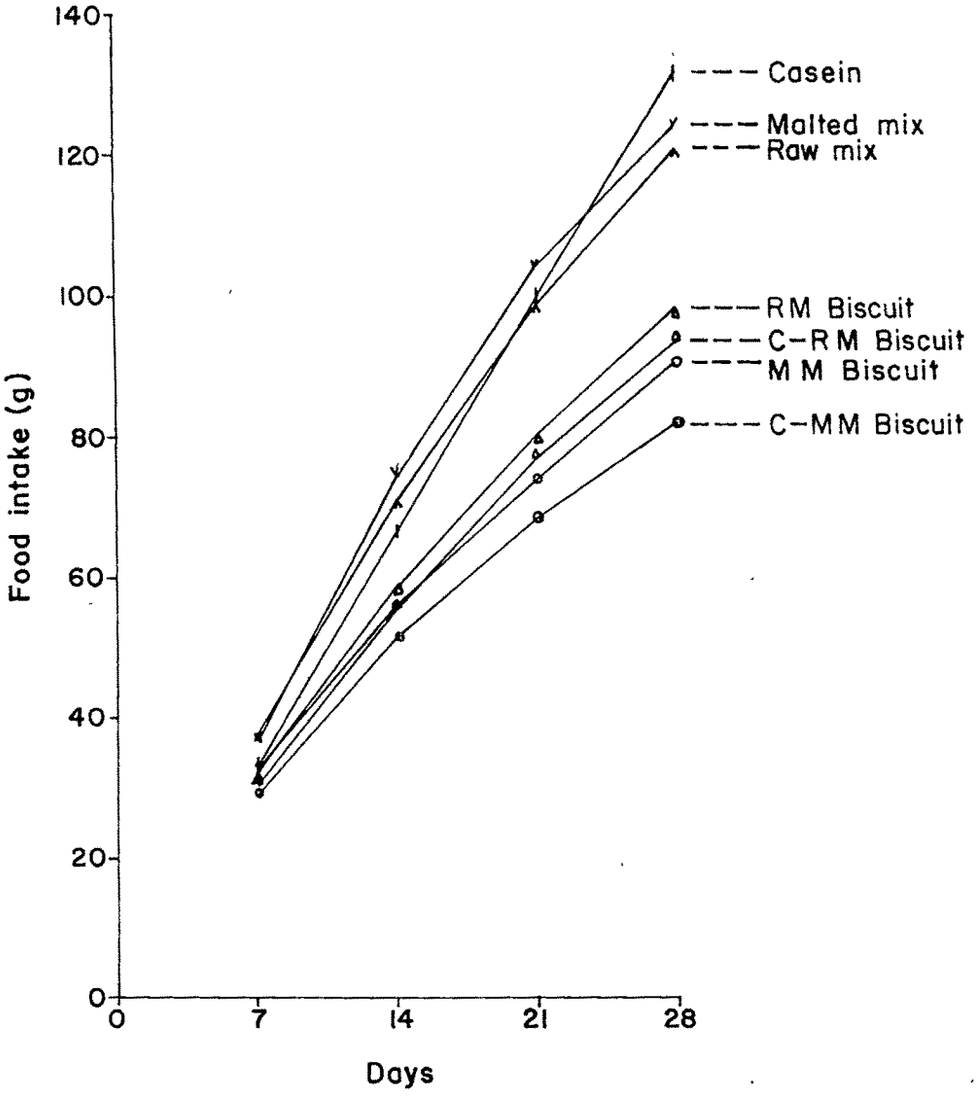
lost significantly more weight and ate less than those fed diets containing mixes (Table 65). The rats fed biscuit diets began to eat less than those fed diets containing mixes from the seventh day of the experiment (Fig 16). Thereafter a progressive decrease in food intake was recorded with the result that at each weekly interval the gap in food intake between the biscuits or mixes diets fed rats became progressively wider. Within the biscuits and mixes fed groups, however, no marked differences were observed in food intake at any given time (Fig 16). The decrease in food intake in biscuit fed group, perhaps, was due to the fact that the biscuit diets were not palatable to the rats and/or that amino acid imbalance caused by baking had led to a loss of appetite and consequently lower food intake (Li and Anderson 1983) which in turn, resulted in decreased growth rate. Recently, Schneeman (1985) has opined that food intake is depressed when heated protein is fed to the experimental animals because the animals try to protect themselves from excess intake of energy. He observed that when heated non fat dry milk (NFDM) was fed to rats they decreased their food intake in comparison to those fed unheated NFDM. The loss of appetite was found to be related to the length of time for which NFDM was heated, 30 or 45 min. The results were attributed to the loss of lysine during heating of NFDM because the addition of lysine to heat treated NFDM reversed the poor growth and food intake. Likewise, Bolze et al (1985) have reported that dietary deficiencies of lysine, methionine and histidine caused marked decreases in feed intake, which did not permit weight gain. Rats fed lysine deficient diet had

Fig 16 Weekly changes in food intake of the biscuits, mixes and casein diets fed rats (6% protein diets)



consumed 7.3 g diet/day and had lost 24 g in 21 days while the control group fed 5 g diet/day had lost 16 g/day thereby indicating that lysine deficiency leads to greater weight loss.

Prabhavathi et al (1974) had shown that the free plasma lysine levels were significantly reduced in rats fed biscuits as compared to the values of those fed unbaked biscuits. Also, there was a marked elevation in the threonine, tryptophan and methionine levels in the plasma of rats fed biscuits indicating that on baking, the amino acid imbalance resulted which was reflected on the plasma amino acid profile. Earlier, Prabhavathi et al (1973) and later Hernandez and Sotelo (1984) had reported that the PER values of the baked product proteins were lower than that of the raw ingredient proteins supporting the fact that baking adversely affected the protein quality of the baked product.

In the present study, inclusion of colocasia leaf powder into the biscuits further increased the loss of weight in rats. The rats fed C-MM and C-RM biscuit diets lost about 3 g more weight than those fed MM and RM biscuit diets (Table 65). But the mean food intake did not differ whether the rats were fed MM or RM biscuit diets with or without colocasia leaf powder. Figure 15 shows that rats fed C-MM biscuit diet weighed less than MM biscuit diet fed rats on seventh day of the experiment and then continued to weigh less as the experiment progressed from seventh to fourteenth to twentyeighth day. At each weekly interval the difference in weights between the C-MM biscuit and MM biscuit diet fed rats increased. It was 1.6 g on the seventh day, 1.7 g

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on the fourteenth day, 2.2 g on the twentyfirst day and 3.3 g on the twentyeighth day. On the other hand, C-RM biscuit diet fed rats trailed closely behind RM diet fed rats until 21 days of the experiment and then exhibited a sudden loss in weight as the difference in weights was 1.5 g on the twentyfirst day and 3.0 g on the twentyeighth day. Figure 16 shows that at no point of time there were marked variations in food intakes between C-RM and RM biscuit diets fed rats yet there was a weight loss which suggests that there might be some component in colocasia leaf powder which caused growth arrest. However, the C-MM biscuit diet fed rats began to eat less than MM biscuit diet fed rats from the fourteenth day of the experiment and continued to eat less until the end of the experimental period. This might partly explain the higher loss of weight in C-MM biscuit diet fed rats as compared to C-RM biscuit diet fed rats. Hence it seemed that the inclusion of colocasia leaf powder did not improve the growth promoting ability of the biscuits although 7.5 g of colocasia leaf powder provided additional 54 mg of lysine. Similar results were reported by Phansalkar et al (1957). The authors had evaluated the protein quality of a mixture containing wheat, bengal gram and amaranth providing 6, 3 and one percent protein, respectively, in a 10% protein diet and had observed that the PER value of the amaranth mixture protein (2.19) was comparable to that (2.18) of the wheat and bengal gram (7:3, protein) mixture protein thereby suggesting that addition of amaranth had not improved the protein quality of cereal-pulse mixture. Vaidehi (1983) had also shown that the addition of carrot leaf powder

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to a nutri-mix did not improve its PER value.

Within the colocasia containing biscuit diets fed rats, those fed C-MM biscuit diet lost more weight in comparison to those fed C-RM biscuit diet (Table 65). The higher loss of weight perhaps was due to the fact that the C-MM biscuit diet fed rats ate significantly less than those fed C-RM biscuit diet. Figure 15 shows that on the seventh day of the experiment the mean weight of the C-RM biscuit diet fed rats was higher by 1.7 g than that of those fed C-MM biscuit diet although there was a negligible difference in the food intakes between the 2 groups in this period (Fig 16). Thereafter, the differences in loss of weight and food intake widened with the increase in the experimental period.

The rats fed biscuit diets lost weight and ate significantly less than those fed casein diet. These findings were attributed to the variations in the quality of protein.

Table 67 shows that the mean available lysine contents of the biscuits ranged from 1.51 to 1.72 g/16g N. The MM biscuits with and without colocasia leaf powder contained less amount of lysine than that of corresponding RM biscuits but the values were statistically non significant (Table 68). It is worth recalling here, that the available lysine contents of malted and raw mixes were 3.61 and 3.42 g/16g N, respectively. The lower values for available lysine of biscuits in relation to mixes indicate loss of lysine as a consequence of baking.

Table 67. Protein quality of biscuits and casein diets

Groups	Available lysine g/16g N	NPU %	BV %	DC %
		Mean ± SE		
MM biscuits	1.52 ±0.035	55.6 ±5.344	74.4 ±5.089	74.0 ±2.638
RM biscuits	1.72 ±0.270	49.6 ±5.759	71.8 ±7.495	69.0 ±3.255
C-MM biscuits	1.51 ±0.050	44.9 ±2.678	77.6 ±2.164	57.9 ±3.364
C-RM biscuits	1.56 ±0.300	45.7 ±3.351	81.7 ±5.076	55.8 ±1.820
Casein	-	79.0 ±2.602	88.4 ±3.103	89.4 ±1.818

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Table 68. 't' values for the variables of Table 67

Comparisons	Available lysine	NPU	BVv	DC
Among biscuits				
MM Vs RM biscuit	0.735 NS	0.764 NS	0.287 NS	1.193 NS
MM Vs C-MM biscuit	0.164 NS	1.790 NS	0.579 NS	3.766**
RM Vs C-RM biscuit	0.396 NS	0.585 NS	1.094 NS	3.540**
C-RM Vs C-MM biscuit	0.164 NS	0.186 NS	0.743 NS	0.549 NS
Between biscuits and casein				
MM biscuit Vs casein	-	3.937**	2.349*	4.806***
RM biscuit Vs casein	-	4.653***	2.046 NS	5.472***
C-MM biscuit Vs casein	-	9.132***	2.855*	8.237***
C-RM biscuit Vs casein	-	7.850***	1.126 NS	13.064***

See Table 63 for foot note

The values for available lysine contents of the biscuits observed in the present study were found to be lower than those reported by Clegg (1960) for biscuits containing groundnuts with 8 and 15% skim milk powder (1.79 and 2.07 g/16g N, respectively).

In the present study jaggery was used as the sweetening agent in the preparation of biscuits. To examine whether jaggery provided reducing sugars which could augument the maillard reaction during baking, jaggery was analysed for the reducing sugars and was found to contain 11.1 g reducing sugars/100 g. It is possible that the free amino acids present in the malted mix might have reacted with the reducing sugars of jaggery resulting in undigestible compounds. Wolf et al (1981) had demonstrated that maillard browning was the major pathway accounting for free methionine and free lysine losses in the presence of reducing sugars. Such losses could have been of lesser magnitude when biscuits were prepared from raw mix because the contents of free amino acids in raw mix were expected to be relatively lower than those in the malted mix. However, the available lysine content of MM biscuit although lower (1.52 g/16g N) was not significantly different from that of the RM biscuit (1.72 g/16g N) yet it was observed that the rats fed MM biscuit diet lost more weight (about 5g) than those fed RM biscuit diet. A similar observation has been made by Boctor and Harper (1968). The authors had observed that egg albumin autoclaved with one to 2% glucose decreased the availability of lysine as measured by the fluorodinitrobenzene (FDNB) method but the rat

growth assay had given a far lower value. This lowering was found not to be due to the formation of toxic compounds nor to the loss of methionine but to the increased excretion of lysine and other amino acids in feces. The authors were of the view that some of the FDNB available lysine might have been excreted as part of the undigested residue in the feces. Likewise, Donoso et al (1962) found that the protein value of the heated material as shown by bioassay fell by a greater extent than could be accounted for by losses of lysine, available lysine or the sulphur amino acids. Balance studies of nitrogen, sulphur and lysine carried out concomitantly had shown that the fall in the utilization of lysine was largely accounted for by a reduction in its digestibility whereas the falls in the utilization of nitrogen and sulphur were mainly due to a reduced retention of the absorbed elements.

Mori and Nakatsuji (1977) had reported that the nutritive value of casein browned by amino-carbonyl reaction with glucose at 37°C was low due to the formation of a lysine derivative in the protein, which remained in the small intestinal lumen as an absorption delayed material and was finally excreted in the urine.

The protein quality of MM biscuits in terms of NPU, BV and DC tended to be superior than that of RM biscuits although the differences in the values were not statistically significant (Table 67). But surprisingly rats fed MM biscuit diet lost more weight than those fed RM biscuit diet (PER experiment) inspite of their food intakes being comparable. It could perhaps be due

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to the fact that in PER studies, the rats remained on test diets for a longer period of time in comparison to those in NPU, BV and DC studies (28 Vs 10 days).

Incorporation of colocasia leaf powder to MM (from 74.4 to 77.6%) and RM biscuits (from 71.8 to 81.7%) improved the mean BV values of the biscuit proteins. Earlier, Talwalker and Patel (1970a) had also observed improvement in BV values when jowar protein was supplemented with methi (from 79 to 89%) protein. But the mean NPU values of MM and RM biscuit protein with the inclusion of colocasia leaf powder tended to be lower. The mean NPU values decreased from 55.6 to 44.9% when colocasia leaf powder was incorporated into MM biscuits and from 49.6 to 45.7% when colocasia leaf powder was incorporated into RM biscuits.

Likewise, the mean DC values were significantly reduced when colocasia leaf powder was incorporated into MM (from 74.0 to 57.9%) and RM (from 69.0 to 55.8%) biscuits. A decrease in nitrogen digestibility (from 84.2 to 79.8%) was also observed by Adrian and Peyrot (1971) when sorghum was supplemented with cassava leaf powder. In the present study, the decreases in digestibility of the protein of the biscuits containing colocasia leaf powder were attributed to the increase in fibre content of the biscuits, as the fibre content of the biscuits containing colocasia leaf powder was 50% higher than that of the biscuits without colocasia leaf powder (1.49 Vs 0.99 g fibre/100 g biscuits). The decrease in digestibility coefficient as a consequence of

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fibre content of the product has been reported by Ranhotra et al (1971). The authors reported that the digestibility of wheat protein concentrate containing 3% fibre was 83.3% and that of wheat ground shorts containing 10% fibre was 70.9%. However, Mitaru and Blair (1984) had concluded from their results that cellulose did not have a detrimental effect on the dietary protein digestibility but lignin and/or some other undetermined factors in the hulls of rapeseed and soya bean had an adverse effect on protein digestibility. Longe (1984) had demonstrated that rats fed plant fibres of okra fruit (Abelmoschus esculentus), African mango (Irvingia gabonesis) and cedar (Telostemon manli) seeds exhibited decreases in weight gain and food conversion efficiency and increases in fecal nitrogen excretion indicating apparent low nitrogen digestibility. However, Nomani and Stansberry (1982) had observed that wheat bran reduced the nitrogen digestibility but the PER values were not affected while pectin did not reduce the nitrogen digestibility but the PER was significantly reduced. In the present study, the fibre of colocasia leaf powder might have influenced the digestibility of protein.

Comparing the mean NPU, BV and DC values of the C-MM and C-RM biscuit proteins it was observed that the NPU (44.9 Vs 45.7%) BV (77.6 Vs 81.7%) and DC (57.9 Vs 55.8%) values of C-MM biscuit protein and those of C-RM biscuit protein were not significantly different from each other (Table 68). However, the mean NPU, BV (except for RM and C-RM biscuits) and DC values of the biscuit proteins were significantly lower than those of the casein protein.

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The mean hepatic protein content of the rats fed MM biscuit diet tended to be higher than that of the RM biscuit diet fed rats (Table 69). Between the biscuits and mixes diets fed rats, the mean hepatic protein contents of those fed biscuit diets were not markedly different from those fed mix diets (Table 69).

Addition of colocasia leaf powder to the MM and RM biscuits exerted a beneficial effect on hepatic protein contents as the mean hepatic protein contents of rats fed C-MM and C-RM biscuit diets were higher than that of those fed MM (16.6 Vs 15.2 g/100 g liver) and RM (15.8 Vs 14.4 g/100 g liver) biscuit diets but it was significantly elevated only in the latter group of rats (Table 70). Talwalkar and Patel (1970b) had placed rats on diets containing jowar alone (5% protein) and jowar (3% protein) with methi or ambadi (2% protein, each) leaf powder for 9 days after they had been on protein free diet for 3 weeks. The authors observed that the hepatic protein contents were significantly elevated in methi and ambadi supplemented groups over the pre-experimental levels (pre-experimental levels of liver protein were determined after 3 weeks on protein free diet). Likewise, Sehgal et al (1975) had reported higher hepatic protein contents of rats fed diets containing wheat flour supplemented with mustard, spinach and raya leaf powders in comparison to that of those fed wheat flour. Shukla and Sur (1978) had also observed that the hepatic protein levels of rats fed diets of wheat and rice supplemented with cauliflower leaf powder were higher than that of rats fed diets of wheat and rice without cauliflower leaf powder.

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Table 69. Hepatic protein, serum protein and serum urea contents of rats fed biscuits, mixes or casein diet

Groups	Hepatic protein g/100 g	Serum protein g/dl	Serum urea mg/dl
		Mean ± SE	
MM biscuits	15.2±0.632	5.01±0.226	60.41±3.025
RM biscuits	14.4±0.269	5.10±0.098	55.57±3.658
C-MM biscuits	16.6±0.578	5.03±0.102	69.79±2.998
C-RM biscuits	15.8±0.536	5.20±0.117	61.08±3.060
Malted mix	15.0±0.616	5.20±0.172	21.86±2.981
Raw mix	15.4±0.725	4.84±0.070	29.46±5.586
Casein	14.4±0.502	6.14±0.177	23.26±2.138

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Table 70. 't' values of the variables of Table 69

Comparisons	Hepatic protein	Serum protein	Serum urea
Among biscuits			
MM Vs RM biscuit	1.164 NS	0.366 NS	1.020 NS
MM Vs C-MM biscuit	1.636 NS	0.081 NS	2.202*
RM Vs C-RM biscuit	2.333*	0.654 NS	1.155 NS
C-MM Vs C-RM biscuit	1.015 NS	1.097 NS	2.033 NS
Between biscuits and mixes			
MM biscuit Vs malted mix	0.227 NS	0.669 NS	9.077***
RM biscuit Vs raw mix	1.294 NS	2.167*	3.910**
C-MM biscuit Vs malted mix	1.893 NS	0.850 NS	11.336***
C-RM biscuit Vs raw mix	0.443 NS	2.647*	4.965***
Between biscuits and casein			
Malted mix Vs casein	0.755 NS	3.806**	0.382 NS
Raw mix Vs casein	1.134 NS	6.842***	1.037 NS
MM biscuit Vs casein	0.991 NS	3.937**	10.030***
RM biscuit Vs casein	0.000	5.148***	7.626***
C-MM Vs casein	2.872*	5.441***	12.637***
C-RM Vs casein	1.907 NS	4.432***	10.131***

See Table 63 for foot note

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The mean hepatic protein contents of rats fed C-MM and C-RM biscuit diets were found to be comparable with each other (Table 69).

When the mean hepatic protein contents (Table 69) of the rats fed biscuits and casein diets were compared it was observed that although the values of hepatic protein content of rats fed MM biscuit, RM biscuit and C-RM biscuit diets were comparable to that of the casein diet fed rats but that of C-MM biscuit diet fed rats was significantly higher than that of the casein diet fed rats (Table 70).

The mean serum protein contents of the biscuit diets fed rats ranged from 5.01 and 5.20 g/dl (Table 69). The comparisons made within the biscuit diets fed rats exhibited no significant differences (Table 70) between the mean serum protein levels of the rats fed these biscuit diets. The beneficial effect of addition of colocasia leaf powder in biscuits observed on hepatic proteins was not seen on the serum protein levels. These findings are supported by those of Phansalkar et al (1957) who had observed no improvement in serum protein levels on addition of amaranth to cereal-pulse combinations. Shukla and Sur (1978) had also reported that the serum protein levels were not elevated when wheat was supplemented with radish or cauliflower leaves.

When the mean serum protein levels of biscuit diets fed rats were compared with those of the mix diets fed groups it was observed that the serum of rats fed biscuit diets contained as

much protein as that of those fed diets containing mixes (Table 69). The mean serum protein contents of the biscuit diets fed groups were significantly lower than that of the casein diet fed group (Table 70).

The mean serum urea levels (Table 69) of the MM biscuit diet fed rats did not significantly differ from those of the RM biscuit diet fed rats (Table 70). Inclusion of colocasia leaf powder into the biscuits did not alter the protein quality of biscuits as no differences were observed in mean serum urea concentrations between C-MM and MM biscuit diets fed rats. Likewise no variations were observed between the mean serum urea concentrations of C-RM and RM biscuit diets fed rats. Comparing the mean serum urea concentrations of biscuits and mixes diets fed rats it was observed that the serum urea concentrations of the former groups were significantly elevated in comparison to those of the latter groups (Table 70).

In the present study, because the rats fed C-MM and C-RM biscuit diets lost about 3 g more weight than those fed MM and RM biscuit diets, another experiment was planned to investigate whether the ill effects on growth were caused by some toxic component that was present in colocasia leaf powder. The colocasia leaf powder was incorporated into the malted and raw mix diets. Two groups of 6 rats each were fed diets containing malted or raw mix plus colocasia leaf powder. The composition of diets was similar to those of malted and raw mix diets (6% dietary protein level) as presented in Table 58 except that 7.5% colocasia leaf powder was incorporated at the cost of the mixes. The diets were

fed for 28 days. It was observed that the rats fed C-malted mix and C-raw mix diets lost significantly more weight and ate significantly less than those fed malted mix and raw mix diets (Table 71). These data indicated that perhaps colocasia leaf powder has some appetite depressor which ultimately leads to decreased food intake and consequently reduced weight gain.

The magnitude of the adverse effect on weight gain as a result of inclusion of colocasia leaf powder into raw mix was smaller than that observed when colocasia powder was incorporated into malted mix. The decreased food intake, and weight loss in rats fed mixes plus colocasia leaf powder as compared to that of those fed mixes without colocasia leaf powder could be due to the presence of an inhibitory factor in colocasia leaf powder as stated earlier.

When the growth rate of rats fed diets containing C-MM and C-RM biscuits, and C-malted and C-raw mixes was compared it seemed that the rats on colocasia containing mixes diet grew better than those fed colocasia leaf powder containing biscuits. However, comparing the weight change in C-malted mix and C-MM biscuit, and C-raw mix and C-RM biscuit diet fed rats it was observed that the biscuit diets fed rats lost more weight than the mix diets fed rats although their food intakes were significantly higher.

In nutshell, it appeared that (a) the protein quality of malted mix protein in terms of NPU and BV was superior to that of the raw mix but the PER values did not differ between malted

Table 71. Weight change and food intake of rats fed mixes and biscuits

Groups	Weight change (g)	Food intake (g)
	Mean ± SE	
Malted mix	+9.0±0.962	124.6±12.576
C-malted mix	-5.8±1.918	67.92+5.319
C-MM biscuit	-8.9±1.302	82.1±3.262
Raw mix	+8.7±2.130	121.1±8.562
C-raw mix	-2.2±1.419	78.2±2.590
C-RM biscuit	-4.0±0.968	93.9±4.184
Comparisons	't' values	
C-malted mix Vs malted mix	6.873***	4.151**
C-malted mix Vs C-MM biscuit	1.337 NS	2.276*
C-raw mix Vs raw mix	4.259**	4.796***
C-raw mix Vs C-RM biscuit	1.048 NS	3.190**

See Table 63 for foot note

and raw mix proteins. These data did indicate that malting tended to improve the protein quality of the grains, (b) heat treatment to the mixes in biscuit preparation lowered the growth promoting ability of the mixes, (c) although the NPU, BV and DV values of MM biscuit protein were higher than that of the RM biscuit protein, the rats fed MM biscuit lost more weight which perhaps was due to the fact that in PER studies, the rats remain on the test diets for a longer period of time as against in NPU, BV and DC studies (28 Vs 10 days), (d) the incorporation of colocasia leaf powder did not improve the growth promoting ability of the biscuits and (e) an inhibitory factor in colocasia leaf powder might be present.