FURTHER STUDIES ON THE NUTRITIONAL ASSESSMENT OF GHEE-RESIDUE PROTEINS AND THEIR AMINO-ACID CONTENT

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

Master of Science

IN

DAIRYING

(HUMAN NUTRITION AND DIETETICS)

TO THE KURUKSHETRA UNIVERSITY

KURUKSHETRA

1980

By

Kusum Malhotra

DIVISION OF HUMAN NUTRITION AND DIETETICS

NATIONAL DAIRY RESEARCH INSTITUTE

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This is to certify that the thesis entitled "Further studies on nutritional evaluation of ghee residue protein and its amino acid composition" submitted in partial fulfilment of the requirement of the degree of Master of Science, in Human Nutrition and Dietetics, of the Kurukshetra University, is a compilation of genuine research work carried out by Miss Kusum Malhotra (B.Sc. Home Science) under my guidance and supervision.

No part of this thesis has been submitted for any other purpose elsewhere. All the assistance and help received during the course of investigations has been duly acknowledged here.

S.N.Ghosh 27.9.80

(S.N.GHOSH)

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(KUSUM MALHOTRA)

I AFFECTIONATELY DEDICATE THIS WORK

TO

MY PARENTS

C O N T E N T S

INTRODUCTION

INTRODUCTION

From times immemorial it is universally known to all strata of people that milk and its products are highly nutritious food items, much before the nutritional merits of milk were established scientifically. Researches conducted in the field of nutrition during the last few decades, have revealed the presence of several nutrients of high quality in milk which explained its many health giving properties.

Milk, the natural food of young mammals contains very high quality proteins and calcium that meet their essential nutritional requirements. Milk protein occupies a pivotal position in human nutrition. Milk in addition provides considerable quantities of various other essential nutrients such as calcium, phosphorus, certain vitamins of B-group and vitamin A.

Indian vegetarian diets are highly deficient in good quality animal proteins. By adding certain amount of milk to the diet this deficiency can be met considerably, but its per capita availability in India is very low.

Generally, total production of milk in India is about 23 million tonnes per year. Out of which 44.5% is consumed as fluid milk, 33% is converted into ghee, 6% to butter, 8% to curd, 5% to khoa, 2% to cream, 1% to other products.

Ghee is the most important dairy product in India because it possesses a good taste and very attractive aroma. So naturally it is extensively used in diet preparations for imparting good taste and imparting aroma. It is widely used in religious ceremonies also in India. It is a rich source of energy, fat soluble vitamins, some amount of essential fatty acids and has sufficiently long shelf life ant room temperature.

When milk is converted into different products through various processes, some by-products are naturally produced which contain a large proportion of one or more of the original constituents. Generally many of these by-products are allowed to go as waste. Not much interest is taken for their utilisation. If proper steps are taken to utilise them, 773 million kgs of milk solids could become available for supplementing the diet of the people if given at the rate of about 30 gm/day/head (i.e. 10.5 kg/head/year) will help in reducing the deficiency of diets of about 71 million people per year. Fourteen percent of the total solids of milk is utilised for the manufacture of common indigenous products. The solids of the milk get distributed in products and by-products. Quite a large portion of these solids goes to the by-products which is mostly being wasted unutilised.

Serious steps are therefore called for to utilise the by-products as these are nutritious and can be used to supplement the poor Indian vegetarian diet. This will lead to the improvement of health, well being and reduce cases of protein deficiency. From the dairy industry in the country, large quantities of skim milk, butter milk, cheese-whey, ghee residue and other by-products are available.

Among these by-products in this country, the solids residue obtained in ghee making is more amendable to further exploitation, since by virtue of its physical character, its easy collection and centralised handling it can be taken up further processing for possible useful utilisation.

Ghee residue is the solid that remains at the bottom of the pan as a brown sediment during clarification process and this sediment on filtration and maximum possible extraction of fat, is ghee residue. It contains caramelised milk sugar, heat affected milk proteins, varying amount of milk fat and minerals. It has been reported that ghee residue contains about 23% protein, 35% fat and 6% ash etc.

Relwani (1978) had tried minor supplementation with small quantity of lysine to improve the nutritive

quality of ghee residue protein but could find little improvement, in the nutritional response of the animals probably due to inadequate lysine dose.

In 1979 Grewal observing some amount of supplementary effect of lysine assumed that probably a larger dose of lysine could show better nutritional performance of rats and took up studies with 4% and 8% dose of lysine in place of 0.5% dose used by Kelwani. She also used some other essential amino acids individually to study their effect, if any, on the improvement of the ghee residue protein. She got encouraging response by employing higher doses of lysine supplementation, whereas with other essential amino acids namely methionine, tryptophan she observed slight improvement, as compared to that produced by lysine incorporation in the diet.

Further to study the effect of another essential amino acid, threonine and also the combined effect of those amino acids used in earlier study, the present study was undertaken. In addition, effort has also been made to study the amino acid make-up of ghee residue protein. The results are delineated in this thesis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Heat treatment is an important and indispensable step milk has got to be subjected before its consumption. The heat induced changes, as a result of heat treatment on milk proteins are of great importance in the manufacture of milk products. Knowledge of the heat induced changes on proteins of milk is very important, since it acts as a guide to formulate the suitable time-temperature combination for manufacturing milk products of certain desirable qualities. Many investigators have made concentrated efforts to elucidate the heat induced changes in the constituents of milk. These changes are mostly irreversible. Although heat treatments bring about changes in the constituents of milk resulting in phenomena like cooked flavour, development of antioxygenic properties, prolongation of rennet clotting time, inhibition of gelation of evaporated milk on sterilisation and finally in the heat stability of milk. Although heat stability is a complex phenomena, its study is made more complex as a result of the interplay of a number of reactions taking place during heat treatments.

Milk proteins are the most important constituents as far as heat stability of milk is concerned. A number of changes namely, aggregation, denaturation, coagulation

and interaction between various proteins takes place as a result of heat treatments.

Effect of Heat Treatment on Distribution of Nitrogen

As a result of heat treatment especially at temperature higher than 65°C, considerable changes take place in the distribution of nitrogen in the various proteins of milk. Rowland (1934) ascertained the extent of heat denaturation of albumin and globulin in cow's milk at various temperatures from 63 to 80°C for periods ranging from 2½ to 60 minutes. Later Rowland (1937) studied the heat denaturation of albumin and globulin in cow's milk at temperatures varying from 75°C to 120°C. The denaturation of albumin and globulin was rapid in samples of milk heated at a temperature of 75°C and above.

Hetrick and Tracy (1950) studied the effect of high temperature short time treatment on the distribution of nitrogen in milk. These investigators did not observe any denaturation of albumin and globulin when milk was heated to minimum temperature for inactivation of phosphatase enzyme.

Lyster et al. (1971) investigated the effect of direct and indirect method of ultra-high temperature treatment on the distribution of nitrogen. These results indicated considerable increase in casein nitrogen and slight decrease in proteose-peptone nitrogen (PPN).

Santha and Narayanan (1978) studied the effect of temperature and period of heating during the clarification of cream/creamary butter to ghee on the nitrogenous constituents of ghee residue. The soluble nitrogen content of ghee residues obtained by heating cream and creamary butter to 120°C was 0.82% and 0.89% respectively which was found to decrease as the period of heating increased. The average non-protein nitrogen content of creamary butter ghee-residue was 0.81%

Effect of Heat Treatment on Milk Proteins

According to Farah, (1979) during the UHT treatment of milk, an interaction occured between casein and whey protein, leading to the formation of a complex of the two proteins.

Srinivasan and Gopalan (1971) observed that when buffalo milk protein was heated at 110°C for an hour there was a decrease of (2.5%) in most of the amino acids, but when heating was continued for 24 hrs at 110°C, the loss was 50%. If the protein extracted from milk was treated with 0.02% of hydrogen peroxide (H_2O_2) and heated at 110°C for an hour, the loss was 10%. This loss was 67% when milk was preheated with 0.1% hydrogen peroxide.

Out of the heat labile amino acids only lysine was observed to be affected by various heat treatments (Hodson, 1952, Mauron et al., 1955).

Tylkin and Tsaberyabaya (1976) reported that UHT (140°C for 4 sec.) treatment reduced total amino acids by 9.3% with further losses of 1.7% during storage for 5 to 10 days. Essential amino acids content/100 gm of protein was 58.2 gm in raw milk, 53.8 gm after UHT treatment and 53.1 gm after 5 days storage. Maximum losses were observed in lysine 16.3%, cystine 16.0%, glutamic acid 14.5% and methionine 13.1%. A 12% loss of tryptophan was found by Menden and Cremer (1956) by sterilisation of milk. Twenty minutes heating at 100°C - 125°C resulted in a progressive loss of lysine (Schober and Prinz, 1956).

Bruel et al. (1972) used one batch of liquid skim milk heated at 61°C for 20 sec. to prepare four samples of spray processed powder under different heating conditions - viz 72°C for 15 sec., 95°C for 30 sec., 75°C for 30 min. and 80°C for 30 min. They also prepared two samples of roller processed powder from the same batch, one of normal appearance and the other slightly scorched. They found that lysine content was affected only in the second roller dried skim milk sample and its content in the protein was reduced to 77% of the original amount present in the untreated milk. They further demonstrated that the availability of lysine was lower in both the normal and scorched roller dried

samples, being 86 and 64% respectively. In experiments conducted on rats they found that by feeding both the dried skim milk in combination with gluten in order to introduce lysine as the Ist limiting amino acid, both normal and scorched samples of roller dried skim milk yielded lower figure for biological value. Improvements resulted by adding 0.2% to 0.3% lysine respectively to their diets. They concluded that various preheating conditions during processing of spray dried skim milk had no deleterious influence on the nutritive value of protein.

Dworschak (1973) observed that the lysine and tryptophan in dried skim milk powder, showed that the decomposition of lysine followed a 4th order reaction, while that of tryptophan was of the Ist order. He further found that heating at 100°C for 4 hrs there was a 25% reduction in the relative nutritive value as the tryptophan and lysine became limiting.

Ford and Porter (1961) studied the changes found in the availability of amino acids in dried skim milk powder, whole meat meal and fishmeal after dry heating at 105°C for 0.5, 1, 2 and 5 hrs. They found that heating reduced the contents of available lysine, methionine and tryptophan in the materials but the loss of available lysine was relatively much greater in the

heated skim milk powder. Similar view was expressed by Carpenter et al. (1963) who showed by rat feeding test that the effect of heating mixture of buffalo casein and glucose of high moisture content resulted in reduction of available lysine, rather than that of methionine.

Dehaas et al. (1978) studied the effect of processing on lysine availability in milk products. They found that lysine in freeze dried evaporated skim milk and whey was slightly less available than that in instantized dried skim milk. The total lysine of milk product was similar but that of dried skim milk was decreased by 56% by autoclaving at 121°C for 2 - 5 minutes. He further stated that commercial processing of milk products results in minimal reduction in lysine availability.

Huss (1974) explained that at 130°F loss of available lysine after two months storage was 31% to 34% and at 30°C it was about 78% after 7 months.

Hodson (1972) observed that there was no loss of amino acids in evaporated milk after one year's storage at 40°F (5°C) but measurable losses of tryptophan, lysine, histidine, arginine occured after one year's storage at 100°F (38°C). After 2 yrs storage, no losses

of amino acids were observed at room temperature. At 100°F, 12% of tryptophan, 29% lysine, 29% histidine and 28% of arginine were lost. Evaporated milk stored for 27 months at 40°F (5°C), proved markedly superior for rats than that stored at 100°F, for the same period. Samples of evaporated milk stored at 100°F for 2 years had markedly deteriorated in appearance and palatability.

Rose and Krampitz (1960) studied protein breakdown in spray dried skim milk by giving further heat treatments using various temperatures and time combinations from half an hour to 3 hrs. Arginine was most, lysine moderately and histidine was the least affected by heat treatment.

Holsinginer (1979) demonstrated that the excessive or prolonged exposure to heat, during manufacture of dehydrated products from cheese whey and cheese whey protein concentrate was chiefly responsible for decreasing the lysine content of these products. Both total and available lysine were higher in spray dried whey powder of the lysine originally available in the protein of cheese whey. 88% remained available in the experimental dehydrated high protein isolate, unfractionated foam spray dried cottage cheese whey powder retained 96% of original available lysine.

Balden (1951) observed that much of the loss caused in nutritive value was due to destruction of amino acids particularly lysine or arginine. The animal experiment showed that autoclaved casein with glucose, arginine, lysine, methionine, histidine and threonine were unable to help growth. He showed that casein autoclaved with glucose at 121°C for 18 minutes bound the carbohydrate through its free amino acid groups. Further heating produced binding of carbohydrate by condensation around the carbohydrate already bound by amino groups. By increasing the water content of casein glucose mixture this reaction was inhibited to some extent, whereas increasing the temperature of processing had an adverse effect on protein particularly in short time processing.

Mauron and Moltu (1958) verified the specific measurement of the free amino groups of lysine in milk protein. He found that fresh milk contains 8.3% lysine, where lysine availability in enzymic form was 8.3% and with FDNB was 8.0% but roller dried milk contained 6.2% lysine and lysine availability in enzymic form was 2.3% and with FDNB was 4.0%.

Effect of Heat Treatment on Biological Value

Fairbank and Mitchell (1935) found a decrease of about 8% nutritive value of the protein in roller

processed powders than in preheated spray processed powders. For raw and evaporated milk the biological values were 85.0 and 84.6 % respectively, after heating but there was slight change in digestibility.

Further, they stated that as the drying temperature (roller process) increased from low to the scorching temperature, the digestibility of proteins was lowered from 92% to 81% and the B.V. from 89% to 79%. In B.V. test the low temperature products were improved by the addition of cystine but in the slightly scorched products, lysine appeared to have been destroyed indicating that these latter products were of little value in supplementing the cereals. Even with the extreme scorching, the net energy value was not affected.

Smith et al. (1953) demonstrated that digestibility of autoclaved milk and milk powder was almost the same as that of unheated milk but that of evaporated milk was slightly higher. B.V. showed little variation, apart from a slight decrease in samples autoclaved in the laboratory, evaporated and frozen milk led to the greatest nutritive indices, the former from its digestibility and the latter from its unexpectedly high B.V. It was concluded that commercial processing had no deleterious effect on the protein of milk.

Lawrence et al. (1953) reported that there was no decrease in B.V. or D.C., when raw milk, evaporated milk and whole milk powders were fed to the animals. Simulation of processing condition by autoclaving raw milk produced no observable nutritive damage.

Sterilisation of evaporated milk at 115°C to 116°C for 15 minutes caused slight decrease in PER and at 130°C to 140°C there was decrease in D.C. of casein and fall in growth rate of rats; but when lysine was fed with casein the growth was partially restored and when histidine was added it caused additional boost. The growth rate of both heated and unheated lactalbumin improved by supplementing with amino acids like valine, leucine, threonine, methionine and arginine.

Kraft and Morgan (1951) reported that decrease in B.V. was chiefly due to loss of lysine and due to destruction or reaction of free amino groups with aldehyde formation of this and other unnatural linkage not susceptable to normal enzymatic digestion. They observed the effect when milk sample was autoclaved for 15 to 25 minutes at 120°C and fed at 12% protein level to groups of rats alone and supplemented by lysine, methionine, valine or all other amino acids at 1% level. Milk autoclaved for 15 minutes lost from ½ to 2/3 of its

growth efficiency but this was largely restored by lysine or lysine, methionine, and valine supplement. The samples autoclaved for 25 minutes did not support the growth except when lysine supplemented.

Kiiza (1973) reported that progressively increasing destruction of methionine and less markedly cystine with increase in severity of time and temperature combination. The availability of amino acids of milk protein is influenced by the heat treatment, that it undergoes. Kon and Anon (1962) demonstrated only marginal effect of heat treatment on B.V. of raw milk 84%, roller dried 83%, evaporated 82%, by comparing the different methods for observing the availability of lysine.

Various Mechanisms of Reactions Involved in Heat Treatment of Protein

Bjarnason and Carpenter (1969, 1970) examined the effect of heat treatment and reported that the maillard reaction between sugar aldehyde group and the free amino group and the free amino acids of proteins was responsible for much of the heat damage to protein when sugar was present. Protein also suffered heat damage in the presence of sugar or any oxidizing fat which might provide carbonyl groups for a maillard type reaction. Bjarnason (1970) found that heating of protein under severe processing conditions in industry may cause a

marked loss of cystine and a smaller loss of tyrosine and lysine.

The epsilon-amino group of lysine apparently reacted with the amide group of asparagine and glutamine to make the lysine indigestible. Howat (1970) proved that the presence of carbohydrate increased the loss of lysine and caused the loss of arginine even at a temperature around 100°C.

According to Patton, 1955 and Ellis, 1959 that milk acquires a brown colour of varying intensity when subjected to high temperature as in the manufacture of sterilized milk and condensed milk. When condensed and dried products were stored under adverse conditions, this discolouration was due mainly to the interaction of lysine and to a lesser extent of arginine and histidine from casein with lactose to exhibit the maillard reaction. The reaction leading to the formation of the brown pigment melanoidin was autocatalytic and was accelerated by high temperature and humidity. Dutra et al. (1959) observed that in addition to the maillard reaction there might be direct caramelization of lactose, though this was only of minor significance.

The maillard reaction was activated by phosphates, metals and oxygen as well as by the decomposed products of lactose like furfural etc. On the other hand, this

reaction was inhibited by sulphydryl compounds which were produced when milk was subjected to high temperature (Lea et al., 1943). They also found that fluorescent substance was also formed during the decomposition of lactose.

Storrs (1942) observed that heating of milk for 30 minutes above 105°C prolonged the heat stability. Grimbley (1954) reported that browning occured at 80°C and that heating at 90°C and 100°C for 2 hrs, resulted in pronounced denaturation of protein with an increase in number of free amino groups which reacted with lactose and caused the formation of cooked flavour.

Palton and Flipse (1953) observed that part of the heat-produced-complex was unstable to heat which ultimately gave rise to the browning effect due to appearance of a number of decomposition products of lactose. The complex also acted as a catalyst for the further thermal decomposition of lactose.

Andrin (1975) noticed that heating altered the structural composition of casein, by heating, the lysine being unavailable due to complex formation with lactose known as maillard reaction. This phenomenon was highest in milk powders and least in liquid milk because water inhibits maillard reaction.

Frangne et al. (1973) reported that when purified animal and vegetable proteins were subjected to maillard reaction under standard conditions. The percentage of lysine destroyed was proportional to its percentage in the protein and ranged from 40% in albumin and in some globulins to 10% in the gelatin and cereals proteins. High lysine proteins therefore demanded careful heat treatments. Sensitivity of milk product was attributed to high lactose and lysine content. The nature of the protein also affected reactivity of sugars; sucrose caused a loss of 56% lysine from lactalbumin and 5% from soyabean globulin. No absolute classification of sugars and proteins with regard to their reactivity in maillard reaction was possible but intensity of reaction depended on sugar protein association.

Trials on 8 purified proteins indicated that each protein contained a definite proportion of lysine which could be destroyed by the maillard reaction. Ovalbumin and glutenin heated for 20 hrs at 120°C lost 68% and 49% lysine respectively after approximately 10 hrs with no further loss occuring. When previous heating had already destroyed part of lysine, losses were due to maillard reaction alone. A direct proportionality was also found between lysine losses due to maillard reaction and the rate of lysine liberation by enzymic hydrolysis

(pepsin, trypsin). The most maillard sensitive proteins are most easily hydrolysed. These results are attributted to the presence of labile lysine fraction in the protein determining its reactivity.

Studies on the ghee residue has already been conducted on various aspects. Santha (1977) has studied its various fraction of lipids. Relwani in 1978 estimated the proximate principles of ghee residue at 110°C - 120°C. She had also tried some minor supplementation with small quantity of lysine to improve nutritive quality of ghee residue protein. There was only slight improvement in the nutritional response of the animal probably due to the very inadequate lysine dose.

Grewal (1979) after observing the slight improvement of lysine supplementation in Relwani's work assumed that probably by increasing lysine dose, some better result might be obtained and she supplemented the ghee residue with 4% and 8% dose of lysine in place of 0.5% dose used by Relwani. She used some other essential amino acids also individually to study their effect if any on the improvement of ghee residue protein. She got very gratifying results by higher dose of lysine supplementation, whereas other amino acids namely methionine and tryptophan showed some improvement but not of much importance.

The present study has been planned to study further the combined effect of the previous three combined amino acids (lysine, tryptophan, methionine) and as well as effect another essential amino acid threonine. The analysis of amino acids setup of ghee residue protein has also been taken up to unvail its exact amino acid composition.

MATERIALS AND METHODS

MATERIALS AND METHODS

Samples of ghee residue at 100°C were specially prepared to see the effect if any of lower temperature ghee preparation on the quality of protein of ghee residue at The Experimental Dairy of National Dairy Research Institute, Karnal and collected from there in wide mouth large bottles with air tight caps to avoid bacterial contamination. The samples were preserved in the deep-freezer.

Method of Production of Ghee Residue at 100°C from Creamery Butter

The loose butter used for the ghee residue was obtained from the same Experimental Dairy (Dairy Technology Division). The same was manufactured from sweet pasteurized mixed cream (cow and buffalo). The percent butter fat and curd content of the butter was 82.85 and 0.8 to 1.2 respectively. The butter was stored at 5°C for 10 - 12 hrs before conversion into ghee. It was heated in a steam jacketed kettle or vat and converted to ghee at 100°C. As soon as the temperature of melted butter came to 100°C, the time was noted and the same temperature was maintained for 15 - 20 minutes. The ghee after settling was decanted and the residue left was collected in beakers. It was then hanged in muslin cloth in an oven maintained at 50 - 60°C for

half an hr. and then squeezed properly to extract maximum fat. After proper draining, the residue in the cloth was pressed between two flat sheets under heavy pressure to remove maximum extractable fat.

Determination of Moisture

Moisture was determined according to the method as given in Dairy Industry Foundation Laboratory, Manual (1949) for cheese.

Determination of Protein

The protein content of the samples was determined by using the conventional micro-kjeldahl digestion and distillation method as given in A.O.A.C. (1975).

Estimation of Lactose

Lactose content in different samples were determined according to the method described by Folin and Wu (1920), with some modifications. This modification is at the step of precipitation of the proteins from the samples.

Reagents:

(a) Alkaline copper reagent.

(b) Phosphomolybdic acid.

(c) Sodium tungstate 10%.

(d) H_2SO_4 2/3 N.

Procedure:

Three gm of ghee residue sample was ground in pestle and mortar and made into a solution with 20 ml of boiled water. The protein was precipitated by adding 20 ml of 10% sodium tungstate and 20 ml of 2/3 N H_2SO_4 to the solution and was filtered. Out of the 60 ml solution, 2 ml was taken in Folin Wu sugar tube and estimation was continued as per the method-mentioned.

Determination of Ash

This was estimated according to the method prescribed in I.S.I. Bulletin 1165 - 67 for cheese.

Determination of Fat

Fat of ghee residue was determined by the Rose and Gottileb method (I.S.I. Bulletin 3507 (1966) II for cheese.

Protein Efficiency Ratio by Depletion Technique

The experiment was conducted on 40 weaning rats abo ut 25 days old, between the weight range of 30 - 40 gms. On the Ist day animals were weighed in the morning with empty stomach and fed protein free diet. The animals were then regularly weighed twice a week in the morning till completion of ten days. The protein free diet was fed for 10 days. On the 11th day the animals were weighed

again and randomly allotted in five groups. Groupwise they were fed the following experimental diets:

Group I - Skim milk diet i.e. protein supplied through spray dried skim milk powder(SMP).

Group II - Ghee residue diet at 110°C i.e. protein at 10% level from ghee residue prepared at 110°C (G.R. - I).

Group III - Ghee residue at 100°C i.e. protein at 10% level from ghee residue prepared at 100°C (G.R. - II).

Group IV - Diet containing ghee residue - I + 8% total lysine + 2.5% methionine + 1.4% tryptophan.

Group V - Ghee residue - I + 4% threonine.

The protein content was maintained at 10% level including amino acids. Urine and faeces for all animals were collected separately by using special method. At the end of 10th day of test protein diet feeding period, the animals were sacrificed, the liver and blood were collected. Protein was estimated by micro-kjeldahl digestion - distillation method in urine, faeces and liver.

Calculation:

$$PER_D = \frac{\text{Wt. of rats at the end of 10 days test protein feeding period} - \text{Wt. of rats at the end of the depletion period}}{\text{Total protein ingestion}}$$

TABLE - A

Percentage Composition of the Diets Used in PER_D

Ingredient	Protein free diet	S.M.P. diet (10% C.P.)	G.R. diet at 110°C (10% protein)	G.R. diet at 100°C (10% C.P.)	G.R. + Amino acids diet (10% C.P.)	G.R. + Threonine diet (10% C.P.)
S.M.P.	-	27.80	-	-	-	-
G.R.	-	-	46.39	38.31	44.53	45.16
Lysine	-	-	-	-	0.80	-
Methionine	-	-	-	-	0.25	-
Tryptophan	-	-	-	-	0.14	-
Threonine	-	-	-	-	-	0.40
Fat original	-	-	9.74	8.53	9.35	9.48
Fat added	12.00	11.50	2.26	3.47	2.65	2.52
Vitaminised fat	0.50	0.50	0.50	0.50	0.50	0.50
Sucrose	9.00	4.00	4.00	4.00	4.00	4.00
Vit. Mixture	1.00	1.00	1.00	1.00	1.00	1.00
Mineral Mixture	3.00	3.00	3.00	3.00	3.00	3.00
Cellulose	4.00	4.00	4.00	4.00	4.00	4.00
Starch	70.00	48.20	29.11	37.11	29.78	29.94

Skim milk powder contains 35.97% crude protein.
Ghee residue at 110°C contains 21.55% crude protein.and 21% fat.
Ghee residue at 100°C contains 25.75% crude protein and 22% fat.

Preparation of Diets:

The composition of each diet for each group has been shown in Table A.

Biological Assay of Protein Quality with Animals:

In the present study the assays used were :

1. To find the digestibility co-efficient (D.C.) of the respective proteins.
2. To find the biological value (B.V.) of the protein.
3. To find the net protein utilisation (N.P.U.).

Estimation of Available Lysine

This was followed according to the method of Carpenter (1960), which is as follow:

Reagents:

(a) Sodium bicarbonate - 8%.

(b) FDNB - was prepared fresh, daily for every sample. Approximately 0.3 ml of fluorodinitrobenzene (pipetted after warming the bottle) dissolved in 12 ml of ethanol was used.

(c) Standard HCl - 8.1 N.

(d) Standard HCl - 1 N.

(e) Sodium hydroxide solution - 2 N.

(f) Phenolphthalein solution - 1% in 70% ethyl alcohol.

(g) Buffer solution - 19 parts of 8% sodium bicarbonate + 1 part of 8% sodium carbonate. The pH was adjusted to 8.5 with addition of a little acid or alkali.

(h) Methyl chloroformate.

(i) Diethyl ether - free from peroxide.

(j) Standard DNP-lysine hydrochloride monohydrate - 25 mg of DNP-lysine hydrochloride monohydrate was weighed accurately and dissolved in 500 ml of 1 N HCl acid solution (so that 2 ml of this solution contained 39.85 microgram of available lysine).

Procedure:

The procedure was followed in duplicate away from direct or strongly reflected light.

(1) 0.75 gm of sample was weighed accurately into a 100 ml round bottom flask and shaken gently with 8 ml sodium bicarbonate solution for 10 minutes. Then FDNB solution was added and shaken gently continuously for two hours. The ethanol was evaporated off on boiling water bath. Then 24 ml of 8.1 N HCl was added and refluxed gently for 16 hours. After 16 hours the solution was cooled suitably for easy filtration in ice-water for 2 hrs and the contents were filtered with water washings into 200 ml volumetric flask and later the volume was made up to the mark.

(2) 30 ml aliquot of the filtrate was transferred in 50 ml volumetric flask and made upto the mark with 1 N HCl.

(3) 2 ml of the diluted filtrate was transferred to a glass stoppered tube and extracted with 5 ml of diethyl ether each time untill the final ether layer was clear. The last ether layer was sucked with a pasteur pipette and finally removed by keeping the tube in boiling water bath.

(4) After evaporation of ether the volume of the tube was made to 10 ml with 1 N HCl and later extinction was read at 435 mu.

(5) For the blank a second aliquot of 2 ml diluted filtrate was taken in a tube (see No. 2) and extracted with ether as before. Also a 3rd aliquot of 2 ml was taken in a conical flask for a dummy titration. Diluted and added phenolphthelin as indicator and titrated with the 2 N NaOH in burette. The volume of NaOH solution needed was noted and the flask was discarded. The same volume of NaOH was added to the second tube and then 2 ml of buffer solution of pH 8.5 was added (this stage was continued without pause as DNP-compounds are less stable at this pH).

(6) 0.05 ml of methyl chloroformate was added to the second tube. Shaken and allowed to stand for 5 to 10 minutes. Then 0.75 ml concentrated hydrochloric acid was added cautiously to the solution to avoid excessive effervescence. This was again extracted

with 5 ml portion of diethyl ether twice. The extracted ether was removed in a different tube. All residual ether was evaporated out and the volume was made upto 10 ml with water. The extinction of the tube was read at 435 mu.

7. The experiment was to be completed through (point No. 3-6) with standard DNP-lysine also. But the available standard with which this wa to be attempted was not found in order, as it could not be dissolved and so the estimation unfortunately could not be completed. Fresh standard could not be procured inspite of making much efforts to obtain it from many parts of the country.

<u>Calculation</u>

Available lysine gm/100 g of crude protein

$$= \frac{X_1 - X_2}{S_1 - S_2} \times \frac{39.85}{1000000} \times \frac{50}{2} \times \frac{200}{30} \times \frac{100}{W(100-M)} \times \frac{100}{P}$$

Where X_1 = Absorptioneter reading with unknown

X_2 = Blank Absorptiometer read with unknown

S_1 = Absorptiometer reading with std.

S_2 = Blank Absorptiometer reading with Std.

W = Wt. in gm of the material taken.

M = Moisture % by wt. in the material.

P = Crude protein (on dry basis % by wt.

Amino Acid Analysis

Protein hydrolysate was prepared by acid hydrolysis. 100 mg of defatted material was taken in a vial. To this 5 ml of 6 N HCl was added and then hydrolysed for 18 hrs at 110°C in a sealed vial. After hydrolysis the seal was broken and volume was made upto 10 ml and filtered. From the filtrate 2.5 ml was taken and dried at 80°C under vaccum. This dried material was dissolved in 2 ml of buffer solution. From this 250 microlit. of the solution was injected in the automatic amino acid analyser for the analysis of amino acids.

Determination of Tryptophan

Tryptophan was determined by the colorimetric method of Spies and Chamber (1949).

Reagents

1. 19 N H_2SO_4
2. 0.045% Sodium Nitrite solution.
3. Para dimethyl amino benzaldehyde.

Procedure

50 mg of the defatted sample, containing (20 - 100 ug of tryptophan) was taken in three 50 ml conical flask, each marked A, B and C. To the flask A and B, 30 mg of p-dimethyl amino benzaldehyde were added whilst the flask marked C serve as blank. To all the flasks 10 ml 19 N H_2SO_4 were added and the flasks were put in the

dark at 30°C for 18 hours. The flasks were then removed 0.1 ml of sodium nitrite solution was added and the solution shaken gently. The transmittance of the samples were read at 590 nm. The tryptophan content in the sample was found by reference to a standard curve prepared by taking known concentration from 20 - 100 ug of tryptophan after making necessary corrections of blank.

RESULTS AND DISCUSSION

RESULT AND DISCUSSION

Ghee residue is known to be a source of animal protein. Work has been done to study the nutritional quality of ghee residue protein. In present investigation attempts have been made to study further the nutritional quality of ghee residue protein and also to see the affect of temperature of preparation.

Composition of Creamary Butter Ghee Residue

It was seen from Table I and 2 that the average protein content of ghee residue obtained at 110°C (G.R. II) was more as compared to that obtained at 100°C (G.R. I). On the other hand the fat, lactose and moisture contents were more in G.R. I as compare to G.R. II. The values of G.R. II obtained with respect to all chemical comonents i.e. moisture, fat, ash, lactose and protein were found to be similar to that observed by Relwani (1978) and Grewal (1979). A comparison of composition of G.R. II in last three studies is in Table 3.

The lower protein content of G.R. I can be partly explained on the basis of lower processing temperature which resulted in partly higher moisture and much higher lactose content. Lactose content was higher may be because butter obtained was not properly washed.

TABLE 1

Chemical composition of creamery butter ghee residue at 100°C (C.B.G.R.)

S.No.	Moisture %	Fat %	Protein %	Lactose %	Ash %
1.	24.86	38.02	19.10	14.95	4.07
2.	23.76	36.42	17.35	18.85	3.52
3.	24.76	37.18	18.88	15.05	4.54
4.	25.07	34.65	20.41	16.09	3.78
5.	24.27	38.40	18.37	15.90	3.72
6.	23.19	35.45	17.86	20.01	4.02
7.	26.0	38.46	20.92	19.99	3.15
8.	30.56	37.91	15.82	18.4	3.19
M.V.=	25.30	37.06	18.58	17.40	3.74
S.E.=	±0.841	±0.510	±0.619	±0.773	±1.395

M.V. = Mean Value

S.E. = Standard Error

TABLE 2

Chemical composition of creamary butter ghee residue at 110°C (C.B.G.R.).

S.No.	Moisture %	Fat %	Protein %	Lactose %	Ash %
1.	22.00	35.00	25.64	12.84	4.52
2.	24.20	33.89	26.00	11.20	4.69
3.	25.30	34.49	25.80	8.94	5.27
4.	22.90	33.95	24.70	12.84	5.52
M.V. =	23.62	34.33	25.53	11.45	5.00
S.E. =	±0.720	±0.355	±0.501	±0.817	±0.236

TABLE 3

Comparison between the average composition of ghee residue studied by various workers.

Workers	Moisture %	Fat %	Protein %	Lactose %	Ash %
Relwani (1978)	22.58	35.14	23.90	11.13	6.46
Grewal (1979)	23.80	35.69	23.21	11.61	5.21
Present study (1980)	23.62	34.33	25.53	11.45	5.00

Protein Efficiency Ratio by Depletion Method(PER_D)

In order to determine the protein quality of G.R. studies were conducted to find its growth promoting capability and effect on the internal organ weight, using protein depleted albino rats as experimental animals. Previously this experiment was conducted with G.R. and G.R. + individual amino acid supplementation. In present study those amino acids were used together for seeing the combine effect of their supplementation. As seen from the data Table 4 that PER_D for animals fed G.R.II + the amino acids was higher than the other groups receiving ghee residue diets and also with control group.

Animals receiving S.M.P. diet were found to be higher PER_D value than G.R. II + Threonine. PER_D value for G.R. I is slightly higher than G.R. II. Though these values are high but these are more or less similar value. Probably due to lesser adverse effect due to low temperature.

A amino acids with G.R. II exhibited significant improvement with respect to the other groups receiving G.R. II diet alone. The PER_D value was higher as compare to the previous workers. This observed differences could be due to that the particular G.R. sample used might be better in nutrition quality.

TABLE 4

Protein efficiency ratio by depletion method(PER_D)

Diets		No. of animals	Av. gain in wt.	Av. Protein intake	Av. PER_D
S.M.P.	(M.V.)	7	31.92	8.85	3.590
	(S.E.)				±0.087
G.R. at 110°C	(M.V.)	8	22.75	10.09	2.235
	(S.E.)				±0.069
G.R. at 100°C	(M.V.)	8	19.33	7.90	2.264
	(S.E.)				±0.083
G.R. + Amino acids	(M.V.)	8	37.33	9.19	4.106
	(S.E.)				±0.528
G.R. + Threonine	(M.V.)	8	16.5	6.67	2.456
	(S.E.)				±0.516

TABLE 4 a.

Analysis of Variance PER_D

Source of Variation	Degrees of Freedom	Sum of Squares	Mean sum of Squares	F Value	F Tabulated at 5%	T Tabulated at 1%
TREATMENT	4	23.336	5.834	139.904*	2.65	3.93
ERROR	34	1.4195	0.041			
TOTAL	38	24.777	0.652			

* Highly significant at 5% and 1% level.

Critical Difference 7 observations = 0.2644

Critical Difference 8 observations = 0.2473

N.B. Statistically there is a significant differences between S.M.P. diet, G.R. + the amino acids and other diets, so it has significant effect on PER_D,

Statistically there is a significant differences between S.M.P. and G.R. + the amino acids diet on PER_D

G.R. at 110°C and G.R. at 100°C has no significant differences so these have the same effect.

Similarly there is a non-significant difference between both ghee residue diets and G.R. + Threonine.

Biological Value and Digestibility Coefficient

To study intrinsic nutritive value of G.R. alone, B.V. and D.C. was derived from above experiment. Data given in Table 4 showed that B.V. of G.R. II + the amino acids was significantly higher than G.R. I, G.R.II and G.R. II + Threonine, slightly higher to S.M.P. also. G.R. II + Threonine was found to be some what higher with respect to B.V. in comparison to G.R. I and G.R. II. B.V. for G.R. I and G.R. II were found to be more or less the same (83.59% /84.60%).

It is well known that B.V. of protein is affected by the proportion of essential amino acid present it. Although heat treatment of milk affects the lysine. The losses during heating the milk in the limiting sulphur amino acid are of greater concern, than the loss of lysine, for milk has an abundance of lysine that a considerable proportion may be inactivated without a corresponding reduction in B.V. (Rolls and Porter, 1973).

Maillard reaction does not cause reduction in B.V. Milk acquires a brown colour when subjected to high temperature. This discolouration was due mainly to the interaction of lysine and to a lesser

TABLE 5

Biological value

Treatments		No.of animals	Av.total N_2 intake (mg)	Av. (Fn-Fe) (mg)	Av. (Un-Ue) (mg)	B.V. (%)
S.M.P.	(M.V.)	7	1387.14	130.94	90.75	92.171
	(S.E.)					±0.418
G.R. at 110°C	(M.V.)	8	1577.375	188.84	197.14	83.595
	(S.E.)					±1.028
G.R.at 100°C	(M.V.)	8	1237.00	179.61	157.25	84.600
	(S.E.)					±1.420
G.R. + Amino acids	(M.V.)	8	1439.75	108.63	78.40	93.836
	(S.E.)					±0.865
G.R. + Threonine	(M.V.)	8	1044.50	163.34	139.51	86.212
	(S.E.)					±0.839

Fn = Faecal N_2 on test protein diet.

Fe = Faecal N_2 on nitrogen free diet.

Un = Urinary N_2 on test protein diet.

Ue = Urinary N_2 on nitrogen free diet.

TABLE 5 a.

Analysis of Variance B.V.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean sum of Squares	F Value	F Tabulated at 5%	T Tabulated at 1%
TREATMENT	4	762.959	190.739	29.544*	2.65	3.93
ERROR	34	219.528	6.456			
TOTAL	38	982.487	25.854			

* Highly significant at 5% and 1% level.

Critical Difference 7 observations = 2.716

Critical Difference 8 observations = 2.548

N.B. Statistically there is a significant differences between G.R. + the amino acids, S.M.P. and other diets so it has significant effect on B.V.

Statistically G.R. + the amino acids, S.M.P. diet has non-significant difference (between these two only).

G.R. diet at 100°C and G.R. at 110°C has non-significant differences.

TABLE 6

Digestibility coeffcient (Apparent)
(D.C.)

Treatment		No.of animals	Av.total N_2 intake (mg)	Av.total N_2 voided in faeces(mg)	D.C. (%)
S.M.P.	(M.V.)	7	1387.14	177.28	87.16
	(S.E.)				±1.169
G.R. at 110°C	(M.V.)	7	1619.14	264.91	84.75
	(S.E.)				±1.852
G.R. at 100°C	(M.V.)	7	1284.14	212.00	84.16
	(S.E.)				±0.036
G.R. + Amino acids	(M.V.)	7	1450.71	205.05	86.24
	(S.E.)				±1.581
G.R. + Threonine	(M.V.)	7	1061.42	139.64	86.91
	(S.E.)				±1.030

TABLE 6 a.

Analysis of Variance D.C.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean sum of Squares	F Value	F Tabulated at 5%	F Tabulated at 1%
TREATMENT	4	11.610	2.910	0.913	2.69	4.02
ERROR	30	441.750	14.725			
TOTAL	34	453.360	13.334			

Non-significant at 5% and 1% level.

extent of arginine and histidine from casein with lactose to exhibit the maillard reaction (Patton, 1951 : Ellis, 1959). The reaction leading to the formation of the brown pigment melanoidin was autocatalytic and was accelerated by high temperature and humidity.

As regards digestibility co-efficient similar trend was observed with D.C. for ghee residue, being close to that observed for skim milk protein and G.R. II + the amino acids.

Net Protein Utilization

With help of B.V. and D.C., N.P.U. was arrived at by calculation. The data for N.P.U. was shown in Table 7 and 7a.

It can be observed that B.V., D.C. and N.P.U. for G.R. II and G.R. I diet was low as compare to S.M.P. with the supplementation of G.R. II with combine amino acid, N.P.U. was similar as that of S.M.P. and with supplemented G.R. II + Threonine there was slight improvement in N.P.U. Significant differences on N.P.U. obtained with S.M.P. diet, G.R. II + the amino acid were found as compare to G.R. values. Both G.R. II and G.R. I diets has same effect on N.P.U.

TABLE 7

Net protein utilisation (N.P.U.)

Treatments		No.of animals	Biological value (%)	Digesti-bility coeffi-cient (%)	N.P.U. (%)
S.M.P.	(M.V.)	7	92.17	87.16	80.40
	(S.E.)				±1.178
G.R.at 110°C	(M.V.)	7	82.41	84.75	69.72
	(S.E.)				±1.765
G.R. at 100°C	(M.V.)	7	84.91	84.16	71.08
	(S.E.)				±1.543
G.R. + Amino acids	(M.V.)	7	94.24	86.24	80.64
	(S.E.)				±1.944
G.R. + Threonine	(M.V.)	7	85.81	86.91	74.58
	(S.E.)				±1.166

TABLE 7 a.

Analysis of Variance N.P.U.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean sum of Squares	F Value	F Tabulated at 5%	F Tabulated at 1%
TREATMENT	4	725.810	181.452	11.660*	2.69	4.02
ERROR	30	466.830	15.561			
TOTAL	34	1192.640	35.077			

*Significant at 5% and 1% level.

Critical difference = 4.3057

N.B. Statistically there is a significance difference between S.M.P. and other G.R. diets so it has significant effect on N.P.U.

Statistically G.R. + the amino acids, S.M.P. diet has no significant difference so these have same effect or non-significant effect on N.P.U. (between these two)

G.R. diet at 100°C and G.R. diet at 110°C has non-significant effect on N.P.U.

The results obtained from the addition of amino acids have revealed that the supplementation of amino acid increase the nutritive value of ghee residue protein.

Regeneration of Liver

Data on the change in weight of the liver was observed on feeding different diets such as S.M.P. diet, G.R. II diet, G.R. I diet, G.R.II + the amino acids diets, G.R.II + Threonine diet. It was seen that restoration of weights of different groups was more or less proportional to the PER_D value of the diet. Non-significant difference on liver weight on S.M.P. and G.R. II + the amino acids diet. But S.M.P. diet and other ghee residue diets have significant differences on weight.

Restoration of the liver protein with G.R. II + the amino acids found to be highest followed next by values of S.M.P., G.R. II + Threonine, G.R. I and G.R. II.

Amino Acid

The amino acid set up of G.R. was studied and results are shown in Table 9.

Chemical Composition of Kalakand

Kalakand was estimated to find out what is the

TABLE 8

Regenerated Tissue Weight with Mean Value and Standard Error.

Treatment		No. of animals	Liver weight (gm)	Liver protein (%)
S.M.P.	(M.V.)	7	3.090	20.26
	(S.E.)		±0.104	±0.719
G.R. at 110°C	(M.V.)	8	2.166	18.42
	(S.E.)		±0.159	±0.470
G.R. at 100°C	(M.V.)	8	2.226	18.77
	(S.E.)		±0.155	±0.805
G.R. + Amino Acids	(M.V.)	8	3.938	20.51
	(S.E.)		±0.261	±0.259
G.R. + Threonine	(M.V.)	8	2.246	19.33
	(S.E.)		±0.256	±0.574

TABLE 8 a

Analysis of Variance - Regeneration of Liver

Source of Variation	Degrees of Freedom	Sum of Squares	Mean sum of Squares	F Value	F Tabulated at 5%	F Tabulated at 1%
TREATMENTS	4	16.484	4.121	12.280*	2.65	2.39
ERROR	34	11.460	0.337			
TOTAL	38	27.944	0.735			

* Significant at both levels (5% and 1%)

TABLE 8 b

Analysis of Variance - Regeneration of Liver Protein

Source of Variation	Degrees of Freedom	Sum of Squares	Mean sum of Squares	F Value	F Tabulated at 5%	F Tabulated at 1%
TREATMENTS	4	23.935	5.983	1.754	2.65	2.93
ERROR	34	115.933	3.4097			
TOTAL	38	139.868	3.680			

Non significant at 5% and 1% level.

composition and what price is paid because we have an idea of preparing recipe from ghee residue. Samples of Kalakand from different shops were collected and analysed, specially to know the amount of protein present in this product for which every where people have to pay quite a high price for purchasing such a little quantity of protein. It was postulated that the high protein content of ghee residue as is present in Kalakand can possibly be provided to the people at a highly reduced cost, say at about 40% of the price of Kalakand after removing the deficiency of ghee residue protein.

TABLE 9

Amino Acid Composition of Ghee Residue at 110°C.

Name of Acid	Percentage
Lysine	2.25
Histidine	2.99
Methionine	1.77
Leucine	1.85
Isoleucine	1.99
Tyrosine	2.15
Phenylalanine	3.32
Valine	4.00
Cystine	5.64
Alanine	1.72
Proline	4.59
Glutamic acid	9.90
Serine	2.77
Threonine	2.74
Asparatic acid	4.25
Arginine	4.26
Tryptophan	1.92
Glycine	Nil

TABLE

Chemical composition of kalakand

S.No.	Moisture	Fat	Protein	Lactose
1.	26.70	13.32	10.40	17.50
2.	22.60	9.25	11.13	17.50
3.	18.72	8.91	10.99	17.50
4.	25.42	8.72	10.99	15.00
5.	27.30	8.62	10.20	17.50
M.V. =	24.15	9.76	10.75	17.00
S.E. =	±1.579	±0.867	±0.156	±0.499

S U M M A R Y

SUMMARY

It is universally known that there is shortage of good quality protein in the India and specially of animal protein. Ghee residue contains good amount of animal protein. So it was planned to study the nutritive value of ghee residue protein and its amino acids composition, so that it can be utilised as human food and as a cheaper source of animal protein for the people. Samples of ghee residue were prepared specially at the Experimental Dairy, N.D.R.I.,Karnal, at 100°C to study the affect of temperature on its protein. Samples of ghee residue prepared at 100°C (G.R.I) and also at 110°C (G.R. II) were collected from Experimental Dairy, N.D.R.I. and were analysed to compare their chemical composition.

The constituents in G.R.II and G.R.I were protein 25.53% ± 0.335 and 18.58% ± 0.619, moisture 23.62% ± 0.720 and 25.30% ± 0.841, fat 34.33% ± 0.335 and 37.06% ± 0.510, lactose 11.45% ± 0.817 and 17.40% ± 0.773; ash 5.00% ± 0.236 and 3.74% ± 1.395 respectively. From the above figures it can be seen that protein and ash contents in G.R. II were more than G.R. I. But other constituents are higher in G.R. I than in G.R. II.

After studying comparaiive composition biological trials were conducted with G.R. diets (containing 10% crude protein). Five groups were taken and fed the following diets - S.M.P. diet, G.R. at 110°C (G.R. II) diet, G.R. at 100°C (G.R. I) diet, G.R. II + the amino acids (lysine, methionine and tryptophan) diet, G.R. II + Threonine diet to the respective groups i.e. 1, 2, 3, 4 and 5. On 21st day the rats were sacrificed and liver was taken to see the effect of different diets on regeneration of the liver.

In this experiment it was found that PER_D value was highest in Gr. 4 i.e. 4.106, followed by Gr.I i.e. 3.593, Gr.5 i.e. 2.456, Gr. 3 i.e. 2.264 and last one is Gr. 2 i.e. 2.235.

B.V., D.C., N.P.U. values of the diets were calculated. The biological values were 92.17%, 83.59%, 84.60%, 93.83%, 86.21% for Gr. 1, 2, 3, 4, and 5 respectively. PER_D, B.V., D.C., N.P.U. of G.R.II was low as compared to S.M.P. but when G.R. was supplemented with combined amino acids there was striking increase in these values in relation to that of G.R. and slightly better even in relation to the value of the control group (indicating thereby probably some of the amino acids are partially affected in S.M.P. preparation).

Which were statistically highly significant in relation to G.R. values. But when G.R. II was supplemented with threonine there was slight increase in this value (non-significant).

To study further the qualities of ghee residue protein its amino acid spectrum was also studied, which have been presented inside, showing lysine to be 2.25% which is less as compare to fresh milk protein.

It is seen that the particular ghee residue sample used in this study was probably of a little better quality as compared to the usual ghee residue samples used earlier.

The higher values of PER_D, B.V., D.C., N.P.U. of this sample of G.R. as compared to the figures found in earlier studies, tend to suggest the above inference having no other alternative.

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