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The Effect of Ultrafiltration on Protein Quality of Skimmilk and Cottage Cheese

Rita Y. Y. Tung
Utah State University

THE EFFECT OF ULTRAFILTRATION ON PROTEIN QUALITY OF SKIMMILK AND COTTAGE CHEESE

by

Rita Y.Y. Tung

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Rita Y.Y. Tung

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The Effect of Ultrafiltration on Protein
Quality of Skimmilk and Cottage Cheese

by

Rita Y. Y. Tung, Master of Science

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Major Professor: Dr. Deloy G. Hendricks
Department: Nutrition and Food Sciences

Protein quality in freeze-dried skimmilk (SM), regular cottage cheese (RCC), retentate (Ret) and cottage cheese made from ultrafiltrated skimmilk (UFCC) were evaluated by chemical (amino acid score) and biological methods. Biological evaluation was at 5, 8 and 11% protein level in growing rats by measuring biological value (BV), net protein utilization (NPU) and nitrogen efficiency for growth (NEG) over a 14-day period. A 28-day protein efficiency ratio (PER) was determined on the same products at 10% protein level. Effects of added lactose on PER of retentate, regular and UFCC were also evaluated. The most limiting amino acids were cystine + methionine. Amino acid score for Animal Nutrition Research Council (ANRC) reference casein, SM, RCC, Ret and UFCC was 0.72, 0.91, 0.87, 0.91 and 0.98 respectively according to Food and Agricultural Organization/World Health Organization (FAO/WHO) pattern and 0.45, 0.56, 0.54, 0.57 and 0.61 respectively according to whole egg pattern. PER was 2.7, 3.0, 2.7, 3.1 and 2.8 for

ANRC reference casein, SM, RCC, Ret and UFCC respectively. PER for retentate and skimmilk were significantly different from the cottage cheese. No significant difference in protein quality was obtained when the products were fed at 5, 8 and 11% levels. Average BV was 93, 91, 91, 95 and 94 for ANRC reference casein, SM, RCC, Ret and UFCC respectively. Average NPU was 87, 84, 83, 85 and 85 for ANRC reference casein, SM. RCC, Ret and UFCC respectively. Average NEG values were 66, 73, 70 77 and 73 for ANRC reference casein, SM, RCC, Ret and UFCC respectively. PER values were 2.7, 3.0, 2.7, 3.1 and 2.8 for ANRC reference casein, SM, RCC, Ret and UFCC respectively. Addition of lactose to a level equal to that in skimmilk reduced the PER value of RCC, Ret and UFCC by about 4%, 6% and 4% respectively. Though no significant difference in protein quality of the products were obtained, there was a tendency of ultrafiltration to increase protein quality.

(79 pages)

INTRODUCTION

Dietary proteins differ widely in amounts of each of the eight essential amino acids they contain. Hence, the nutritive value of proteins differ from one another. The protein quality of whole cow's milk is high primarily because milk contains the eight essential amino acids in a ratio compatible with rapid growth.

The proteins in cow's milk can be grouped broadly into 1) caseins and 2) whey proteins. Separately, the nutritive value of casein in whey proteins is different due to the relative concentrations of the essential amino acids; for instance, tyrosine and phenylalanine are comparatively high in casein, cystine and methionine are higher in whey proteins (Walstra and Jenness, 1984). Though several biological indices of protein quality for caseins and whey proteins have been reported, caseins have an average estimate of biological value (BV) of 80 and protein efficiency ratio (PER) of 2.5. The BV for whey protein is 85-98 and PER is 3.0. However, an average BV of 90 and PER of 3.2-3.4 have been reported for whole milk (Walstra and Jenness, 1984).

During the conversion of skimmilk into cottage cheese curd, the caseins are retained in the curd and the whey proteins lost. Hence, the nutritional value of dry cottage cheese curd is lower than that of skimmilk at the same level of protein intake. Any manufacturing process aimed at trapping whey proteins into cheese curd will not only benefit the processor by increasing the yield but will also increase protein quality of the product. Current innovations in dairy technology allow milk to be concentrated by ultrafiltration prior to cheese manufacture. Increased yields obtained from 3 to 5 times concentrated retentate is due to trapping of whey proteins in the cheese structure (Ernstrom, 1986).

The objective of this study was to determine the effects of ultrafiltration on protein quality of the proteins in the retentate and cottage cheese made from it.

REVIEW OF LITERATURE

Methods of Protein Evaluation

Protein is a constituent of every living cell and is vital in the regulation of body processes. After the needs for growth and repair have been met, any remaining protein is used as a source of energy. The efficiency with which a protein is used for growth or maintenance is a measure of its quality. Protein quality is estimated first by its amino acid composition (FAO/WHO, 1973). Other methods used to estimate protein quality are protein efficiency ratio (PER), biological value (BV), net protein utilization (NPU), nitrogen balance incex (NBI), nitrogen growth index (NGI), relative nutritive value (RNV) and most recently nitrogen efficiency for growth (NEG).

Amino acid score

Amino acid score or chemical score is a non-biological method of estimating protein quality. The method compares the amino acid composition of a test protein to that of a high quality or reference protein. Only essential amino acid levels are compared. The essential amino acid with the lowest score may be used as an estimate of protein quality of the food. (Mitchell and Block, 1946; Harper et al., 1955). Irwin and Hegsted (1971) suggested that in addition to essential amino acids, non-essential amino acids must be adequate at the site of synthesis.

Good correlations exist between chemical scores and biological tests (Block and Mitchell, 1946); hence, Rubin(1972) suggests the use of chemical scoring of proteins for product quality control in the food industry because it is relatively faster than biological methods. However, chemical scoring is limited in that true absorption and utilization of protein is only assumed (Guilbault and Hieserman, 1968). Problems of sensitivity, precision and accuracy (Guilbault and Hieserman, 1968) have been reduced with gas-liquid chromatography (Kaiser, et al., 1974). However, there are limitations in estimation of some amino acids like tryptophan and cystine. Accuracy in the estimation of these amino acids depends on the method of hydrolysis used.

Protein efficiency ratio

Protein efficiency ratio (PER) is the major protein quality test specified by the USDA and FDA for use in the food industry (Association of Official Analytical Chemists-AOAC, 1980). It usually involves a 4 week ad libitum feeding of diets containing 10% protein to rats after a 3 to 7 day acclimation period (AOAC, 1980). Several factors have been reported to affect PER assay, eg. age of rats, length of assay period, protein level and sex. Thought standardization of the above factors yields uniform results. PER determinations have been criticized on the basis that (a) gain in body weight may not be constant in composition for different proteins, and (b) the determination makes no allowance for maintenance requirements. The use of a non-protein control group allows for necessary measurement of obligatory nitrogen losses and the use of ANRC reference casein serves as a standard for comparison.

Keane et al.(1961, 1963) showed that water added to a casein diet affects PER values. They observed increased PER values as water content of the diets increased from 0 to 35%. They reported major differences in PER values in the range of 0 to 15% added water. Bender (1956) showed that PER was closely correlated to food intake, and Sure (1955) observed that PER values obtained after 10 weeks feeding of a test protein are lower than values obtained after feeding for 4 weeks.

Biological value

Biological value was first described by Karl Thomas in 1909. It is defined simply as the body's utilization of the end products of protein digestion, hence expressed as a "percentage of the absorbed nitrogen retained by the body for repair or the construction of nitrogenous tissue." (Mitchell, 1923b, p.908). Biological value can be determined only when a test animal is depleted of nitrogen and when intake of protein is low. This allows for adequate measure of utilization of the protein.

Assessment of biological value of proteins and protein mixtures is complicated because of variations in protein concentration. Mitchell (1923 a) assessed the biological value of proteins from corn, milk and oats, and potatoes at 5% and 10% protein levels. With the exception of potatoes, he reported lower BV at higher dietary protein levels. Contrary to this, Henry and Don (1957) did not observe any change in BV when rats were fed diets containing 4 and 8% level of protein from egg and casein. These authors concluded that any decrease in BV in relation to the percent protein in the diet may be a function of the test protein which may be deficient in certain amino acids essential for growth of rats. Hence, the authors suggested that tests using 8% protein in the diet are more reliable tests for the relative nutritive value of proteins than tests conducted using lower levels of protein.

Net protein utilization

Net protein utilization (NPU) represents the portion of food nitrogen retained and is related directly to dietary nitrogen intake. NPU was first described by Bender and Miller (1953) and is used in nitrogen balance studies. Miller and Bender (1955) reported NPU values of 82.0 for dried whey, 75.0 for dried milk and 60.0 for crude casein. All diets were fed at a 10% protein level. Since NPU involves measurement of body nitrogen, factors such as caloric intake, and protein concentration affect values obtained. NPU values are not affected by strain of test animal, water content of food and the number of test animals per cage (NAS-NRC, 1963). Values obtained for NPU depend on the level of protein in a diet and may be affected by caloric intake. However NPU is constant when determined at protein levels below that required for maintenance (NAS-NRC, 1963). For human subjects, NPU determinations are influenced by psychological stress, minor infection, pattern of activity and overall composition of the diet (Pike and Brown, 1984).

Other methods of evaluating protein quality

Several other methods for evaluating protein quality have been used by different researchers to express opinions and views on various facets of protein quality. Hegsted and Chang (1965) developed a slope-ratio bioassay using gain as a response and nitrogen intake as a measure of dose. They described a relative nutritive value (RNV) which is the response of a test protein compared to a reference standard. Usually α-lactalbumin or casein is used as the standard. The potency of this assay is proportional to regression coefficients relating dose and response. Further communications on the slope-ratio assay by Hegsted and Worcester (1966) showed that weight gain, carcass weight, carcass water or carcass nitrogen can be used as a measure of response. Though they were uncertain about the most appropriate and useful response parameter, they reported that carcass nitrogen was of the most interest. A simple linear regression equation relating body protein and protein intake for lactalbumin resulted in a regression coefficient of 0.911 which corresponds to a NPU of 91 (Hegsted and Worcester, 1966).

Allison (1955) described nitrogen balance index as the slope of a line when nitrogen balance is plotted against absorbed nitrogen. This method of evaluating protein quality
is essentially the same as biological value and is calculated as

$$\text{Nitrogen Balance Index} = \frac{B-B'}{A}$$ where N is nitrogen

balance, B' is nitrogen balance at zero nitrogen intake and is absorbed nitrogen. Essentially it is a measure of dietary nitrogen retained and is equivalent to NPU (Bodwell, 1977). A relationship between gain in carcass N and N intake was described by Bodwell (1977) which is termed nitrogen growth index (NGI). This is similar to nitrogen balance index since it measures the amount of dietary nitrogen retained.

A mathematical relationship measuring nitrogen efficiency for growth (NEG) was described by Mahoney et al. (1975) as follows:

$$NEG = \frac{\text{Final carcass N} - \text{Initial carcass N}}{\text{N intake} - \text{Fecal N}} \times 100$$

This method involves estimation of initial carcass nitrogen for each animal by determining the quantity of nitrogen per gram body weight of a number of randomly selected rats at the start of the experiment. The reliability of this method, of course, depends on the accuracy of estimation of initial carcass N, and does not take into account maintenance factors. Any inaccuracies in estimating initial carcass nitrogen is bound to create erroneous results and deviations from expected values. Hendricks et al. (1977) reported NEG for casein 49, and Allred (1976) reported an NEG of 65.6 for casein.

Protein Evaluation of Milk and Cheese

Milk proteins

Nutritionally milk proteins are of high quality. About 77-80% of milk proteins are caseins (Cerbulius and Farrell,1975). The caseins are a group of phosphoproteins in milk which precipitate upon acidification to pH 4.6. The major caseins in bovine milk are α_{S1}, α_{S2}, β and κ-caseins. Apart from the caseins, milk contains a diverse group of proteins which remain in solution at pH 4.6. This rather diverse group includes α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulins and small molecular weight peptides derived by proteolysis of some of the caseins (Walstra and Jenness, 1984).

Cottage cheese

Conventional cottage cheese curd is obtained from rennet-coagulated skimmilk in which pH of the skimmilk is reduced to pH 4.6 by chemical acidification or by metabolism of lactose by lactic acid bacteria. During manufacture, the rennet-coagulum which is mainly a casein-micellar network is cut. The cut curd expells whey consisting mainly of lactose, minerals, vitamins and proteins soluble in water. These proteins include α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulins and the macropeptide portion of Kappa casein. Hence, conventional cottage cheese curd consist mainly of α_{S1}, α_{S2}, β and para-κ-casein.

With the advent of current technology, dairy manufacturers are able to concentrate milk solids by ultrafiltration before cheese manufacture. Data on the nutrient content of cottage cheese manufactured from ultrafiltered skimmilk is not currently available in the literature. Cheeses made from ultrafiltrated milk probably contain more whey proteins and may have higher protein quality.

Protein quality of milk and cheese

The nutritive value of milk proteins depends primarily on their content of the essential amino acids. It is known that the digestibility of milk proteins and absorption of their amino acids is high. Mitchell and Beadles (1950) reported a low BV of 51 for casein, determined with adult rats fed 2 and 4% protein diets. Osborne and Mendel (1924) reported an average BV of casein to be no higher than the protein of corn which has a BV of 72 at 5% level of intake. For casein alone, a PER of 2.5 has been reported (Walstra and Jenness, 1984). The nutritional value of whey proteins alone is greater than that of casein. The BV for whey protein is 85-90 and PER is 3.0. Hegsted and Chang (1965), compared protein utilization in growing rats at different levels of intake. They reported a relative nutritive value of casein, soy protein and wheat gluten to be 0.7, 0.34 and 0.14 relative to α-lactalbumin, a whey protein. Teply et al. (1958) compared the nutritive value of cheese produced from milk treated with hydrogen peroxide and catalase by feeding rats milk, whey and cheese diets containing 9 and 14% protein (dry-weight basis). They observed no differences in nutritive value of milk whey and cheese obtained from the same milk.

There is no doubt that nutritive loss of proteins occurs during heat treatment and processing. Nutritive loss of proteins occurring during heat treatment is due, for the most part, to reactions between the amino acids and a reducing sugar. A significant loss of lysine, arginine, tryptophan and histidine occurs when purified casein is heated in a 5% glucose solution for 24 h at 96.5°C (Patton, et al., 1948). Mader et al. (1949) conducted extensive nitrogen balance studies on dogs fed heated and unheated α-lactalbumin. They reported no change in nutritional quality when commercial lactalbumin was dry heated at 120°C for 60 min. However they found that digestibility and nutritive index were significantly decreased by autoclaving. A digestibility of 95% and nutritive index of 103% was reported for lactalbumin dry heated 120C for 60 min. whereas autoclaving for 60 min reduced digestibility to 72% and nutritive index to 80% (Mader et al., 1949).

Rationale

Current innovations in dairy technology allow milk to be concentrated by ultrafiltration prior to cheese manufacture and yield increases of about 3-5% have been reported (Ernstrom, 1986). Cheese yield increases resulting from the use of UF milk are attributed to trapping of whey proteins into the cheese curd (Ernstrom, 1986). The whey proteins are lost during conventional cheese manufacture (Van Slyke and Price, 1952). The whey proteins (α-lactalbumin, β-lactoglobulin, and bovine serum albumin) are richer in the sulfur containing amino acids than casein which is the major protein in milk and cheese (Walstra and Jenness, 1984). The nutritive value of whey proteins alone is greater than casein. A biological value (BV) of 85-90 and protein efficiency ratio (PER) of 3.0 have been reported for whey proteins(Walstra and Jenness, 1984). The BV and PER for casein are 72 and 2.5 respectively (FAO/WHO, 1973). Hegsted and Chang (1965) reported a relative nutritive value of 0.7 for casein compared to α-lactalbumin. The digestibility of milk proteins and absorption of their amino acid are high, however it is not known whether ultrafiltration of milk affects the protein quality of its products during processing. Therefore the objectives of this study were to determine the effects of ultrafiltration on the protein quality of skimmilk and products made from skimmilk.

MATERIALS AND METHODS

Test Samples

Pasteurized skimmilk was obtained from the USU Dairy Products Laboratory. Retentate (portion of milk retained by the ultrafiltration membrane), regular cottage cheese and cottage cheese manufactured from retentate herein called ultrafiltration cottage cheese (UFCC), made from the same lot of skimmilk were prepared by Mr. Jorge Ocampo, Department of Nutrition and Food Science, Utah State University, Logan. Skimmilk was 3x concentrated to give retentate. Both regular and UF cottage cheese were manufactured by direct acidification of skimmilk and retentate respectively. Initial acidification of the skimmilk and retentate was at 4°C with 85% phosphoric acid to pH 5.2. This was followed by further acidification with glucono-delta lactone to pH 4.7-4.8. The curds obtained after acidification were cut and cooked to an equal temperature of about 54°C. Whey was drained from the cooled curd which was then washed twice with water and the cheese was packaged (Appendix A).

All samples were frozen at -60°C in a blast freezer and freeze dried to a constant weight. The freeze-dried samples were ground and used in preparation of diets. ANRC reference casein was obtained from Nutritional Biochemicals, Cleveland, Ohio.

Test Diets

Experiment 1

Test diets were formulated with the freeze-dried skimmilk (SM), regular cottage cheese (RCC), retentate (Ret), cottage cheese from the retentate (UFCC) and reference casein. Diets from each product were formulated to contain 5, 8 and 11% protein. The total mineral content in the sample required to give the desired protein concentration was calculated. This was based on ash determination in the products. Hence, enough mineral mix was added to constitute 5%, corn oil was 8%, cellulose was 1% and vitamin mix was 1%. Hence enough glucose was added to give the desired final weight of diet (AOAC, 1980). The desired final weight of diet was calculated based on the assumption that each rat consumes 12g/day. Hence, to feed 6 rats for 14 days, 1300g of diet was made with each product (Tables 1, 2 and 3). These diets were fed to rats for determination of BV, NPU, NEG; nitrogen balance index (NBI) and nitrogen growth index (NGI) were also determined.

Experiment 2

Two sets of diets were made. The first set was made from each product and contained 10% protein, minerals (5%), corn oil (8%), cellulose (1%) and vitamins (1%) (Table 4).

Table 1. Formulation* of 5% protein diet.

	Ref.casein	SM	RCC	Ret	UFCC	NP
Protein source,g	75.5	180.6	75.5	112.1	74.5	<1.0
Mineral mixture,g[a]	65.0	51.0	63.0	56.9	63.3	65.0
Corn oil,g	104.0	102.2	102.2	101.9	101.9	104.0
Vitamin mixture,g[b]	13.0	13.0	13.0	13.0	13.0	13.0
Cellulose,g	13.0	13.0	13.0	13.0	13.0	13.0
Glucose,g	1029.5	940.3	1033.2	1003.1	1034.3	1105.0
Total,g	1300.0	1300.0	1300.0	1300.0	1300.0	1300.0
Cal/g[c]	3.9	3.6	3.9	3.8	3.9	4.1
% protein[d]	5.02	5.05	5.10	5.01	5.06	5.03

SM = Skimmilk RCC = Regular cottage cheese Ret = Retentate UFCC = Ultrafiltration cottage cheese NP = Non-protein

* See Appendix B for sample calculations.

[a] See Appendix C for complete listing.

[b] See Appendix D for complete listing.

[c] By calculation.

[d] By analysis.

Table 2. Formulation of 8% protein diet.

	Ref.casein	SM	RCC	Ret	UFCC
Protein source,g	120.9	288.6	120.9	179.3	119.2
Mineral mixture,g[a]	65.0	42.6	62.0	52.0	62.4
Corn oil,g	104.0	101.1	101.0	100.6	100.6
Vitamin mixture,g[b]	13.0	13.0	13.0	13.0	13.0
Cellulose,g	13.0	13.0	13.0	13.0	13.0
Glucose,g	984.1	841.6	990.0	942.1	991.8
Total,g	1300.0	1300.0	1300.0	1300.0	1300.0
Cal/g[c]	3.8	3.4	3.8	3.6	3.8
% Protein[d]	8.03	8.10	8.07	8.05	8.01

SM = Skimmilk RCC = Regular cottage cheese Ret = Retentate
UFCC = Ultrafiltration cottage cheese NP = Non-protein

[a] See Appendix C for complete listing.
[b] See Appendix D for complete listing.
[c] By calculation.
[d] By analysis.

Table 3. Formulation of 11% protein diet.

	Ref.casein	SM	RCC	Ret	UFCC
Protein source,g	165.3	396.5	166.3	246.5	164.3
Mineral mixture,g[a]	65.0	34.3	60.8	47.3	61.4
Corn oil,g	104.0	100.1	99.8	99.5	99.3
Vitamin mixture,g[b]	13.0	13.0	13.0	13.0	13.0
Cellulose,g	13.0	13.0	13.0	13.0	13.0
Glucose,g	939.7	743.1	947.1	880.8	949.0
Total,g	1300.0	1300.0	1300.0	1300.0	1300.0
Cal/g[c]	3.7	3.1	3.7	3.5	3.7
% Protein[d]	11.01	11.10	11.01	11.06	10.96

SM = Skimmilk RCC = Regular cottage cheese Ret = Retentate
UFCC = Ultrafiltration cottage cheese NP = Non-protein
[a] See Appendix C for complete listing.
[b] See Appendix D for complete listing.
[c] By calculation.
[d] By analysis.

Table 4. Formulation of 10% protein diet (PER).

	Ref.casein	SM	RCC	RCC+ Lact	Ret	Ret+ Lact	UFCC	UFCC+ Lact
Protein source,g	416.2	1020.6	442.3	442.3	633.6	633.6	427.3	427.3
Lactose,g	--	--	--	367.2	--	340.6	--	361.4
Mineral mixture,g[a]	180.0	100.8	169.6	169.6	134.3	134.3	170.6	170.6
Corn oil, g	277.2	277.2	277.2	277.2	277.2	277.2	277.2	277.2
Vitamin mixture,g[b]	36.0	36.0	36.0	36.0	36.0	36.0	36.0	36.0
Cellulose,g	36.0	36.0	36.0	36.0	36.0	36.0	36.0	36.0
Glucose,g	2643.8	2129.4	2658.9	2291.8	2482.9	2142.4	2654.3	2292.8
Total,g	3600.0	3600.0	3600.0	3600.0	3600.0	3600.0	3600.0	3600.0
Cal/g[c]	3.7	3.2	3.7	3.3	3.5	3.1	3.7	3.3
% Protein[d]	10.05	10.04	10.07	9.94	9.93	10.17	10.09	9.96

SM = Skimmilk RCC = Regular cottage cheese Ret = Retentate
UFCC = Ultrafiltration cottage cheese NP = Non-protein Lact = Lactose
[a] See Appendix C for complete listing.
[b] See Appendix D for complete listing.
[c] By calculation.
[d] By analysis.

Because lactose is lost during ultrafiltration and cottage cheese manufacture, lactose was added to RCC, Ret and UFCC at levels equal to the regular skimmilk diet. All diets in the second set contained 10% protein. Diets from both sets were fed to rats for determination of PER.

Animal Care

Sprague-Dawley weanling male rats (Simonsen Laboratories, Gilroy, Ca.) were used. The rats were allowed a 3 days adaptation to a 12 h light-dark regimen in a temperature controlled room (25.6°C) after which they were weighed and assigned to a dietary treatment. Rats were housed individually in wire metabolism cages equipped to collect feces and urine separately. During the 3 day acclimatization period. rats were fed commercial rat chow.

Experiment 1

Six rats were assigned per treatment group balancing for average weight. To determine initial carcass N content of animals, six randomly selected rats were killed at the start of the feeding trials. Another set of six rats were assigned to a non-protein diet as control. All animals were fed ad libitum for 14 days. Fresh food and deionized water were provided on alternate days. Feces and urine were collected separately for each animal daily. Food spilled or refused was collected and weighed to quantitate food intake. At the end of the feeding period all animals were weighed and killed by decapitation. The carcasses were put into individual canning jars with an equal volume of 5% glacial acetic acid and autoclaved for 1 h at 121°C. The autoclaved carcasses were individually mixed in a waring blendor and samples taken for determination of carcass nitrogen.

Experiment 2

The rats were assigned per treatment group and fed ad libitum for 28 days. Fresh food and deionized water were provided on alternate days. Food spilled and refused was collected and weighed to quantitate food intake. At the end of the feeding period all animals were weighed and killed.

Amino Acid Determination

Freeze-dried samples of skimmilk, regular cottage cheese, retentate and UF cottage cheese were sent to Woodson-Tenent Laboratories, Inc. Memphis, TN. for amino acid analysis. Amino acid composition of ANRC reference casein was supplied by Nutritional Biochemicals, Cleveland, Ohio. From data obtained, amino acid scores were calculated as follows:

$$\text{Amino acid score} = \frac{\text{mg of a.a. in g N of test protein}}{\text{mg of a.a. in g N of reference protein}}$$

The egg protein reference pattern (Pike and Brown, 1984) and also the reference pattern suggested by FAO/WHO (1973) were used for computing amino acid scores.

Nitrogen Analysis

Diet, carcass, urine and fecal nitrogen were determined by Kjeldahl analysis on a Kjeltec Auto analyzer (Kjeltec Auto 1030 analyzer, Tecator Inc., Sweden). Urine and fecal samples were analyzed by macroKjeldahl. One milliliter of urine. 0.5g carcass, 0.5g feces or 0.5g diet was used for the nitrogen determination. All deteminations were in triplicate. The digestion catalyst was 1 Kjeltab (containing 3.5g K_2SO_4 and 0.0035g Se) for the macroKjeldahl and microKjeldahl procedure. A known concentration of HCL (0.0554 N) was the titrating acid. Percent protein in the milk and cheese based diets was calculated as N x 6.38. Data obtained from nitrogen determinations were used to calculate PER, BV, NPU, NEG, NBI and NGI.

Calculation of PER, BV NPU NEG, NBI and NGI

PER was calculated by the formula described by Osborne et al., 1919 where

$$PER = \frac{\text{28 days weight gain (g)}}{\text{28 days protein intake (g)}}$$

BV was calculated as follows (Mitchell. 1924):

$$BV = \frac{\text{N intake} - [(Fn - F0)+(Un - U0)]}{\text{N intake} - (Fn - F0)} \times 100$$

Fn = Fecal nitrogen
F0 = Fecal nitrogen at zero protein intake
Un = Urinary nitrogen
U0 = Urinary nitrogen at zero protein intake

NPU was calculated according to the method of Bender (1956).

$$NPU = \frac{N\ intake - [(Fn - F0) + (Un - U0)]}{N\ intake} \times 100$$

Fn = Fecal nitrogen
F0 = Fecal nitrogen at zero protein intake
Un = Unrinary nitrogen
U0 = Urinary nitrogen at zero protein intake

The procedure described by Mahoney et al. (1975) was used to calculate NEG where

$$NEG = \frac{Final\ Carcass\ N - Initial\ Carcass\ N}{N\ intake - Fecal\ N} \times 100$$

Nitrogen balance index (NBI) was calculated as described by Bodwell (1977) where

$$NBI = \frac{B - B'}{N\ intake}$$

B = Nitrogen balance
B' = Nitrogen balance at zero N intake

Nitrogen growth index (NGI) was calculated as described by Allison (1955) where

$$NGI = \frac{Carcass\ N\ gain\ (g)}{N\ intake\ (g)}$$

Relative nutritive value (RNV) (Hegsted and Chang, 1965) was calculated as

$$RNV = \frac{Weight\ gain\ on\ test\ protein}{Weight\ gain\ on\ reference\ standard}$$

Other Chemical Analyses

Moisture in the freeze-dried test diets was determined in triplicate by drying 2g sample in a forced-air oven at 100C for 24 h. Moisture was taken as weight loss after drying.

Fat in test samples was determined by the Mojonnier modification of the Ross-Gothlieb method (Atherton and Newlander, 1982). Determinations were done in triplicate using 1g of sample.

Ash was determined in triplicate using approximately 10g material. Samples were weighed into a tared 4 inch coors crucible which had been previously heated over a burner for about 5 minutes, then cooled in a desiccator. The tared crucibles plus sample were carefully charred on a hot-plate under a hood and transferred to a muffle-furnace and its contents ashed for 24 h at 560°C. The crucibles were cooled in a desiccator and weighed. Ash was determined using the weight of the residue (AOAC, 1980). Calcium in ashed samples was determined in triplicate by atomic absorption spectrum (AOAC, 1980).

Lactose in the samples was measured in triplicate by the method of Shaffer and Somogyi (1933). Approximately 5g of sample were used for the determinations.

Statistical Analysis

A factorial analysis of variance to show treatment difference due to method, diet, level and any interactions was done. Differences between mean of factors shown to be significant by variance of analysis (ANOV) were determined by the least significant difference(LSD) method (Dowdy and Wearden, 1983). Correlations were obtained between nitrogen intake, weight gain, nitrogen balance and carcass nitrogen gain. Chi-square analysis was done on amino acid composition and amino acid score of the different samples. All statistical analyses were done using the Minitab statistical package.

RESULTS AND DISCUSSIONS

Composition of Skimmilk, Retentate and Cottage Cheese

The composition of the skimmilk, retentate, regular cottage cheese and ultrafiltration (UF) cottage cheese are shown in Table 5. Also shown in Table 5 are the actual yield and yield adjusted to 20% solids for regular and ultra-filtration cottage cheese. An increase in ultrafiltration cottage cheese yield of about 2% over regular cottage cheese was observed when cheese yield was adjusted to equal total solids content.

Table 5. Composition of skimmilk, retentate and uncreamed regular, UF cottage cheese and cheese yield.

Composition	Skimmilk	RCC	Retentate	UFCC
		mean% ± SD		
Protein	3.26±0.03[a]	17.75±0.02	9.20±0.03	17.87±0.01
Fat	0.32±0.02	0.60±0.01	0.39±0.02	0.52±0.01
Moisture	87.14±0.02	79.68±0.03	84.11±0.01	79.08±0.02
Lactose	4.55±0.03	0.80±0.01	4.61±0.02	0.78±0.01
Total solid	8.73±0.02	20.70±0.03	15.80±0.01	22.83±0.02
Actual yield	--	12.63	--	12.58
Adjusted yield (20%)	--	12.20	--	14.36

RCC = regular cottage cheese
UFCC = ultrafiltration cottage cheese
[a]An average of three determinations.

Composition of Test Diets

Mean percent moisture, protein, fat, ash and lactose in in freeze-dried samples of the skimmilk, regular cottage cheese, retentate and UF cottage cheese are shown in Table 6. Also shown in Table 6 is the protein content of the standard casein.

Table 6. Percentage composition of test products.

Item	ANRC Casein	SM	RCC	Ret	UFCC
			mean % ± SD		
Moisture	nd	3.5±0.1[a]	5.0±0.1	3.3±0.1	4.2±0.1
Protein	86.6±0.3	36.4±0.3	86.1±0.4	58.4±0.2	86.7±0.2
Fat	nd	1.0±0.1	2.9±0.1	1.9±0.1	2.5±0.1
Ash	nd	7.8±0.1	2.2±0.1	1.9±0.1	2.5±0.1
Calcium	nd	1.71±0.30	0.25±0.01	0.74±0.03	0.21±0.01
Lactose	nd	51.8±0.8	3.9±0.2	29.6±0.7	3.8±0.4

[a] An average of three test samples
nd = not determined SM = skimmilk RCC = regular cottage cheese Ret = retentate UFCC = ultrafiltration cottage cheese

Amino Acid Composition

Amino acid content (mg/gN) of skimmilk regular cottage cheese, retentate and UF cottage cheese samples is shown in Table 7. Table 7 also shows the amino acid composition of the ANRC reference casein supplied by Nutritional Biochemical, Cleveland, Ohio. Due to difficulties in amino acid analysis and differences in reported amino acid

Table 7. Amino acid compostion of test samples

Amino acid	ANRC casein	SM	RCC	Ret	UFCC
	mg of amino acid per g of N / %				
Trp	94/1.47	89/0.51	83/1.12	90/0.82	86/1.18
Asp	443/6.94	403/2.30	416/5.61	420/3.85	468/6.36
Thr	237/3.71	236/1.35	225/3.04	257/2.36	261/3.55
Ser	313/4.91	310/1.77	343/4.63	331/3.03	361/4.91
Glu	1739/27.27	1088/6.21	1218/16.43	1165/10.67	1105/15.00
Pro	580/9.10	368/2.10	363.4.90	379/3.47	402/5.46
Gly	89/1.40	108/0.62	114/1.54	114/1.05	120/1.63
Ala	153/2.40	189/1.08	175/2.37	203/1.86	195/2.65
Cys	33/0.52	56/0.32	23/0.32	43/0.40	23/0.32
Val	357/5.60	361/2.06	400/5.40	385/3.53	427/5.80
Met	127/1.99	143/0.82	167/2.26	157/1.44	191/2.60
Ile	292/4.58	336/1.92	347/4.68	345/3.16	380/5.17
Leu	521/8.17	595/3.40	609/8.22	604/5.53	646.8.77
Try	301/4.72	217/1.24	338/4.56	275/2.52	339/4.61
Phe	287/4.50	269/1.54	321/4.34	304/2.79	332.4.52
His	158/2.48	173/0.99	189/2.55	183/1.68	195/2.66
Lys	449/7.04	494/2.82	523/7.06	550/5.04	553/7.51
Arg	203/3.18	226/1.29	256/3.46	255/2.34	268/3.64
Total	6376/99.98	5668/32.34	6119/82.49	6067/55.54	6360/86.34
% N CP[a]	--	88.7	95.8	95.0	99.5

[a] Percent N of crude protein accounted for by amino acids.
SM = Skimmilk RCC = Retentate cottage cheese
RCC = Regular cottage cheese UFCC = Ultrafiltration cottage cheese

composition, 10 amino acid values of casein analyzed by 7 different laboratories (Bodwell, et. al., 1981) were compared to values obtained for the products used in this study. Table 8 shows mean, standard error and coefficient of variation of casein reported by Bodwell et. al. (1981). A comparison of the reported levels of 10 amino acids (Table 8) in casein to the levels of the same amino acids in the products used in this study did not show any inaccuracies in the amino acid determination of the products. The percent amino acid content increased during ultrafiltration and cottage cheese manufacture. The percent cystine in skimmilk, regular and UF cottage cheese was essentially similar (0.32 %). Total amino acid content increased during concentration of skim milk and cottage cheese manufacture.

Statistical analysis of amino acid composition of test samples gave an overall chi-square value of 78.98 which is significant ($p<0.05$). This suggests that the total concentration of amino acids in the diets is different.

Processing of skimmilk into regular cottage cheese resulted in loss of cystine. Similarly, the manufacture of cottage cheese from retentate resulted in loss of cystine. This indicates that cystine is lost into the whey during cottage cheese manufacture. Cystine in bovine milk is found in larger amounts in the whey proteins than in the caseins (Walstra and Jenness, 1984). There are 8, 5 and 35 cystine residues per molecule of α-lactalbumin, β-lactoglobulin and bovine serum albumin respectively. κ-

Table 8. Mean, standard error (SE) and coefficient of variation (CV; between 7 laboratories) of ten amino acids in casein.

	Casein		
Amino acid (mg/gN)	Mean	SE	CV%
Ile	335	16	4.8
Leu	635	28	4.3
Lys	528	27	5.0
Met	189	8	4.2
Phe	342	24	7.1
Thr	290	20	7.0
Trp	85	12	14.5
Val	428	19	4.4
Cys	28	5	17.6
Tyr	378	26	6.9

casein and α_{S2}-casein have 2 residues each of cystine, while β-casein and α_{S1}-casein have none. The loss of cystine during cottage cheese manufacture indicates loss of whey proteins. Bastian (1987) reported loss of whey proteins into permeate during ultrafiltration of whole milk.

Amino Acid Score

The quality of a protein may be estimated from its amino acid composition compared to a reference pattern and an ideal protein contains all the essential amino acids in sufficient amounts to meet dietary requirements. Essential amino acid levels in test samples, the FAO/WHO provisional amino acid patterns (FAO/WHO, 1973) and amino acid patterns of whole egg (Pike and Brown, 1984) are compared in Table 9 and 10. The sulfur-containing amino acids (cystine + methionine) were the limiting amino acids. Compared to the FAO/WHO pattern amino acid scores for ANRC reference casein, skimmilk, regular cottage cheese, retentate and UF cottage cheese were 0.72, 0.91, 0.87, 0.91, 0.98 respectively (Table 9). The amino acid scores were 0.45, 0.56, 0.54, 0.57 and 0.61 for ANRC reference casein, skimmilk, regular cottage cheese, retentate and UF cottage cheese respectively when compared to the whole egg pattern (Table 10).

Chi-square analysis at 0.05 level showed that overall amino acid scores for both FAO/WHO whole egg pattern were not significantly different.

Table 9. Essential amino acid levels and scores for skimmilk, regular cottage cheese, retentate, UF cottage cheese and the FAO/WHO provisional amino acid scoring pattern.

Amino acid	Reference casein	SM	RCC	Ret	UFCC	FAO/WHO (1973)
	mg amino acid per g N / amino acid score					
Ile	292/1.17	336/1.35	347/1.39	345/1.38	380/1.52	250
Leu	521/1.18	595/1.35	609/1.38	604/1.37	646/1.47	440
Lys	449/1.32	494/1.45	523/1.54	550/1.62	553/1.63	340
Met+Cys	160/0.72	199/0.91	191/0.87	200/0.91	214/0.97	220
Phe+Tyr	588/1.55	487/1.28	660/1.74	580/1.53	672/1.77	380
Thr	237/0.95	236/0.95	225/0.90	257/1.03	261/1.05	250
Trp	94/1.57	89/1.49	83/1.38	90/1.50	86/1.45	60
Val	357/1.15	361/1.16	400/1.29	385/1.24	427/1.38	310
Total	2698	2800	3041	3014	3242	2215
Amino acid score	0.72	0.91	0.87	0.91	0.97	1.00

SM = Skimmilk RCC = Regular cottage cheese Ret = Retentate
UFCC = Ultrafiltration cottage cheese

Table 10. Essential amino acid levels and scores for skimmilk, regular cottage cheese, retentate, UF cottage cheese and the whole egg provisional amino acid scoring pattern.

Amino acid	Reference casein	SM	RCC	Ret	UFCC	FAO/WHO (1973)
	mg amino acid per g N / amino acid score					
Ile	292/0.86	336/0.99	347/1.02	345/1.01	380/1.12	340
Leu	521/0.96	595/1.10	609/1.13	604/1.12	646/1.20	540
Lys	449/1.02	494/1.12	523/1.19	550/1.25	553/1.26	440
Met+Cys	160/0.45	199/0.56	191/0.54	200/0.57	214/0.61	355
Phe+Tyr	588/1.01	487/0.84	660/1.14	580/1.00	672/1.16	580
Thr	237/0.81	236/0.80	225/0.77	257/0.88	261/0.89	294
Trp	94/0.89	89/0.84	83/0.78	90/0.85	86/0.82	106
Val	357/0.87	361/0.88	400/0.98	385/0.94	427/1.04	410
Total	2698	2800	3041	3014	3242	3060
Amino acid score	0.45	0.56	0.54	0.57	0.61	1.00

SM = Skimmilk RCC = Regular cottage cheese Ret = Retentate
UFCC = Ultrafiltration cottage cheese

The amino acid score (chemical score) of the test samples based on both the FAO/WHO reference pattern (Table 9) and whole egg reference pattern (Table 10) indicate that the sulfur-containing amino acids (cystine + methionine) were the most limiting amino acids. From both patterns, regular cottage cheese had the lowest score (0.72 and 0.45) and cottage cheese from retentate had the highest score (0.98 and 0.61). Increased expulsion of whey during cottage cheese manufacture might be a reason for the lowest amino acid score being observed in regular cottage cheese. Amino acid score for both skimmilk and retentate was similar. The FAO/WHO reported a chemical score of cow's milk as 0.95 (FAO/WHO, 1973). A report of a joint FAO/WHO Ad Hoc expert committee indicated that a knowledge of the entire amino acid pattern is useful in predicting the second and third limiting amino acids. Using the whole egg pattern, the second limiting amino acid in skimmilk and regular cottage cheese was threonine which had scores lower than 100. However, the second limiting amino acid in retentate and cottage cheese from retentate was tryptophan.

Protein Efficiency Ratio and Weight Gain

Weight gain and PER measured at 10% protein level are in Table 11. There was a significant effect ($p<0.001$) of type of diet on PER values (Appendix E). Compared to ANRC reference casein which had a PER of 2.69, PER values

Table 11. Protein efficiency ratio (PER) of test diets and test diets + lactose.

Item	% Protein	Protein intake,g	Weight gain,g mean[a] ± SE	PER	Corrected PER
Ref.casein	10.05	37.66±0.98	101.55±4.06	2.69±0.06	2.5
SM	10.04	35.61±1.16	106.01±3.50	2.99±0.08	2.8
RCC	10.07	38.90±1.12	104.25±3.18	2.69±0.09	2.5
RCC + Lactose	9.94	34.99±0.82	91.98±3.05	2.62±0.04	2.4
Retentate	9.93	38.71±1.28	119.81±3.53	3.11±0.07	2.9
Retentate + Lactose	10.17	38.28±0.79	109.77±3.50	2.86±0.06	2.7
UFCC	10.09	40.75±0.98	113.14±1.42	2.79±0.06	2.6
UFCC + Lactose	9.96	37.77±1.41	101.86±4.17	2.71±0.09	2.5
LSD($p<0.05$)	--	3.06	9.54	0.19	--

SM = Skimmilk RCC = Regular cottage cheese UFCC = Ultrafiltration cottage cheese
a Each mean represents ten observations.

increased in the following order: regular cottage cheese (2.69) < UF cottage cheese (2.79) < skimmilk (2.99) < retentate (3.11). For each test protein, addition of lactose appeared to decrease PER values. A significant decrease (~ 8%) in PER was observed when lactose was added to retentate. PER values decreased by about 3% when lactose was added to either regular or UF cottage cheese. The decreases observed in PER when lactose was added to RCC, Ret and UFCC were not significantly different ($p<0.05$) from the same proteins without addition of extra lactose. Average weight-gain over the 28-day period for rats fed reference casein was 101.55g±4.06. Rats fed retentate gained the most weight (119.81g±3.35). There was a significant effect ($p<0.001$) of type of diet on weight gain of rats (Appendix C). Increasing lactose level in regular cottage cheese, retentate and UF cottage cheese to levels similar to that of skimmilk resulted in a decrease in weight gain compared with original diet. The rats fed retentate + lactose diet gained more weight than the rats fed skimmilk diet (Table 11).

The PER of ANRC reference casein, used as a reference standard was 2.69-0.06. This falls within the range of 2.45 to 3.22 reported by the FAO (1970). The PER of regular cottage cheese (2.69±0.09) was no different from that of casein. This was expected because most, if not all of the protein in regular cottage cheese is casein. The PER for skimmilk was 2.99±0.08. Retentate had a PER of 3.11±0.07 and cottage cheese manufactured from retentate had a PER of

2.79±0.06. The lower PER observed in retentate cottage cheese compared with retentate itself was due to loss of some whey proteins during the cheese manufacture. Hegsted and Worcester (1947) reported that PER is closely related to weight gain regardless of the protein because PER for growth does not consider protein required for maintenance. Differences in food intake affect PER values(McLaughan and Campbell, 1969) and animals with larger food intakes tend to have higher PER (Mitchell, 1924). There was a significant difference ($p<0.05$) in protein intake of rats fed the different diets (Appendix C). However, there was only a low correlation between protein intake and PER ($r=-0.17$).

Rats prefer diets with sweet taste (Winitz et al., 1957) but some carbohydrates may have negative effects on growth and PER values when present in diets at high values (Steinke,1977). Lactose in milk and milk by products may cause gastric intestinal disturbances and diarrhea in rats. Hence, Steinke (1977) suggests that products containing lactose must be tested with a casein control diet containing an equal quantity of lactose. The results of this study showed that addition of lactose to a test diet appeared to decrease PER values. The effects of addition of lactose to regular cottage cheese and UF cottage cheese were not significantly different from the cheese samples alone. However addition of lactose to retentate resulted in a significant lowering of PER (Table 11).

Biological Evaluation

Nitrogen balance data shown in Table 12 indicate that for each test sample, nitrogen absorption over the 14-day feeding period was greater than nitrogen retention during the same period. Mean nitrogen retention was bout 75% for ANRC reference casein, 72% for skimmilk, 73% for regular cottage cheese, 75% for retentate and 73% for UF cottage cheese. Nitrogen absorption was 86% for ANRC reference casein, 82% for skimmilk, 86% for regular cottage cheese, 84% for retentate and 83% for UF cottage cheese.

Figure 1 shows the relationship between nitrogen intake and nitrogen balance for the rats fed the different diets. Significant positive correlations were obtained between nitrogen intake and nitrogen balance. Regression equations relating nitrogen balance and nitrogen intake for rats fed the different diets are shown in Table 13. The regression coefficients of the lines are not statistically different, indicating that the lines are essentially parallel. It has been suggested (Bodwell, et. al. 1981) that the coefficient of regression is equivalent to NPU. The coefficients of regression (nitrogen balance index (NBI)) ranged from 0.80 to 0.83 and are not significantly different from NPU values obtained for the products.

Nitrogen intake and weight gain for rats fed test diets containing 5, 8 and 11% protein are shown in Figure 2. The correlations between nitrogen intake and weight gain were

Table 12a. Mean[a] daily nitrogen retention and nitrogen absorption by rats fed non-protein and reference casein diet.

	Non-protein	Reference casein		
% protein	0	5	8	11
			mean ± SE	
N intake, mg	trace	6.00±0.20	9.50±0.28	14.50±0.35
Fecal N, mg	0.52±0.01	1.00±0.03	1.21±0.06	1.71±0.08
Urinary N, mg	0.31±0.01	0.64±0.02	1.07±0.04	1.50±0.06
N retention, mg	- 0.83±0.02	4.36±0.15	7.22±0.18	11.29±0.21
N retention, %	∞	73±1.00	76±0.92	78±1.00
N absorption, mg	- 0.52±0.01	5.00±0.17	8.29±0.22	12.79±0.27
N absorption, %	∞	83±1.03	87±0.98	88±1.04

[a] An average of six rats.

Table 12b. Mean[a] daily nitrogen retention and nitrogen absorption by rats fed skimmilk and regular cottage cheese diets.

	Skimmilk			Regular cottage cheese		
% protein	5	8	11	5	8	11
			mean ± SE			
N intake,mg	5.01±0.19	8.64±0.21	15.29±0.37	5.29±0.18	9.21±0.32	18.21±0.45
Fecal N,mg	1.14±0.07	1.43±0.09	1.64±0.10	0.93±0.04	1.36±0.06	2.07±0.10
Urinary N,mg	0.86±0.02	0.93±0.02	1.71±0.03	0.64±0.03	1.29±0.04	2.07±0.12
N retention,mg	3.07±0.10	6.28±0.10	11.94±0.24	3.71±0.11	6.56±0.22	14.07±0.23
N retention,%	61±1.02	73±0.99	78±1.00	70±1.05	71±1.00	77±0.99
N absorption,mg	3.93±0.12	7.21±0.12	13.65±0.27	4.36±0.14	7.85±0.26	16.14±0.35
N absorption,%	77±0.98	83±1.01	89±0.99	82±1.02	85±1.05	89±0.98

[a] An average of six rats.

Table 12c. Mean[a] daily nitrogen retention and nitrogen absorption by rats fed retentate and ultrafiltration (UF) cottage cheese diets.

	Retentate			UF cottage cheese		
% protein	5	8	11	5	8	11
			mean ± SE			
N intake,mg	5.57±0.19	9.64±0.26	17.07±0.40	3.86±0.12	11.21±0.32	17.79±0.41
Fecal N,mg	1.00±0.05	1.69±0.07	2.14±0.11	0.93±0.02	1.43±0.08	2.29±0.12
Urinary N,mg	0.43±0.01	0.81±0.02	1.64±0.10	0.50±0.01	1.00±0.02	1.79±0.10
N retention,mg	4.14±0.13	7.14±0.17	13.29±0.19	2.43±0.09	8.57±0.17	13.71±0.19
N retention,%	74±0.99	74±1.05	78±1.00	63±0.98	78±1.03	77±1.01
N absorption,mg	4.57±0.14	7.95±0.19	14.93±0.29	2.93±0.10	9.57±0.19	15.50±0.29
N absorption,%	82±0.97	82±1.04	87±1.05	76±0.99	87±1.01	87±1.02

[a] An average of six rats.

Figure 1. Relationship between nitrogen intake and nitrogen balance for rats fed different test diets.

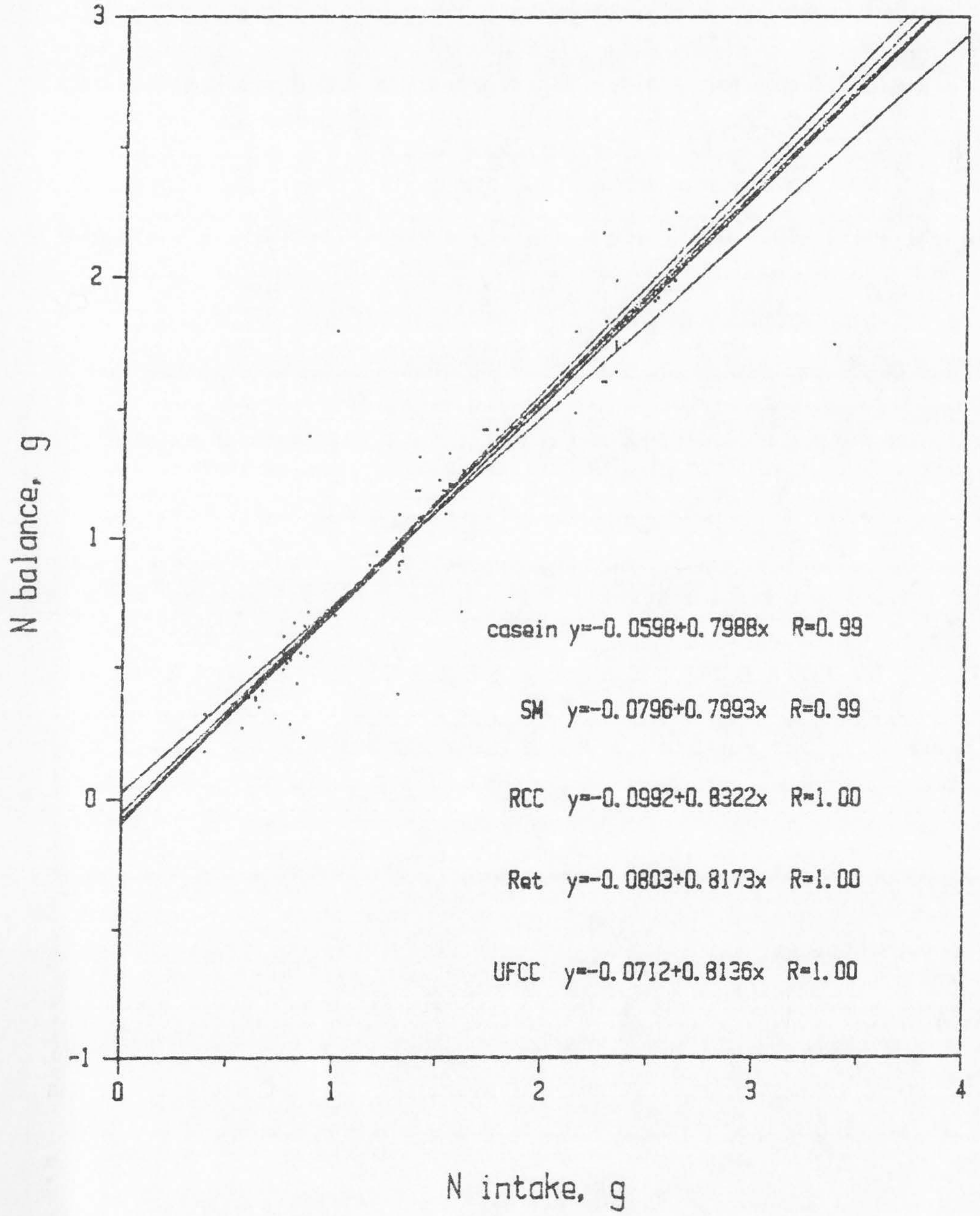
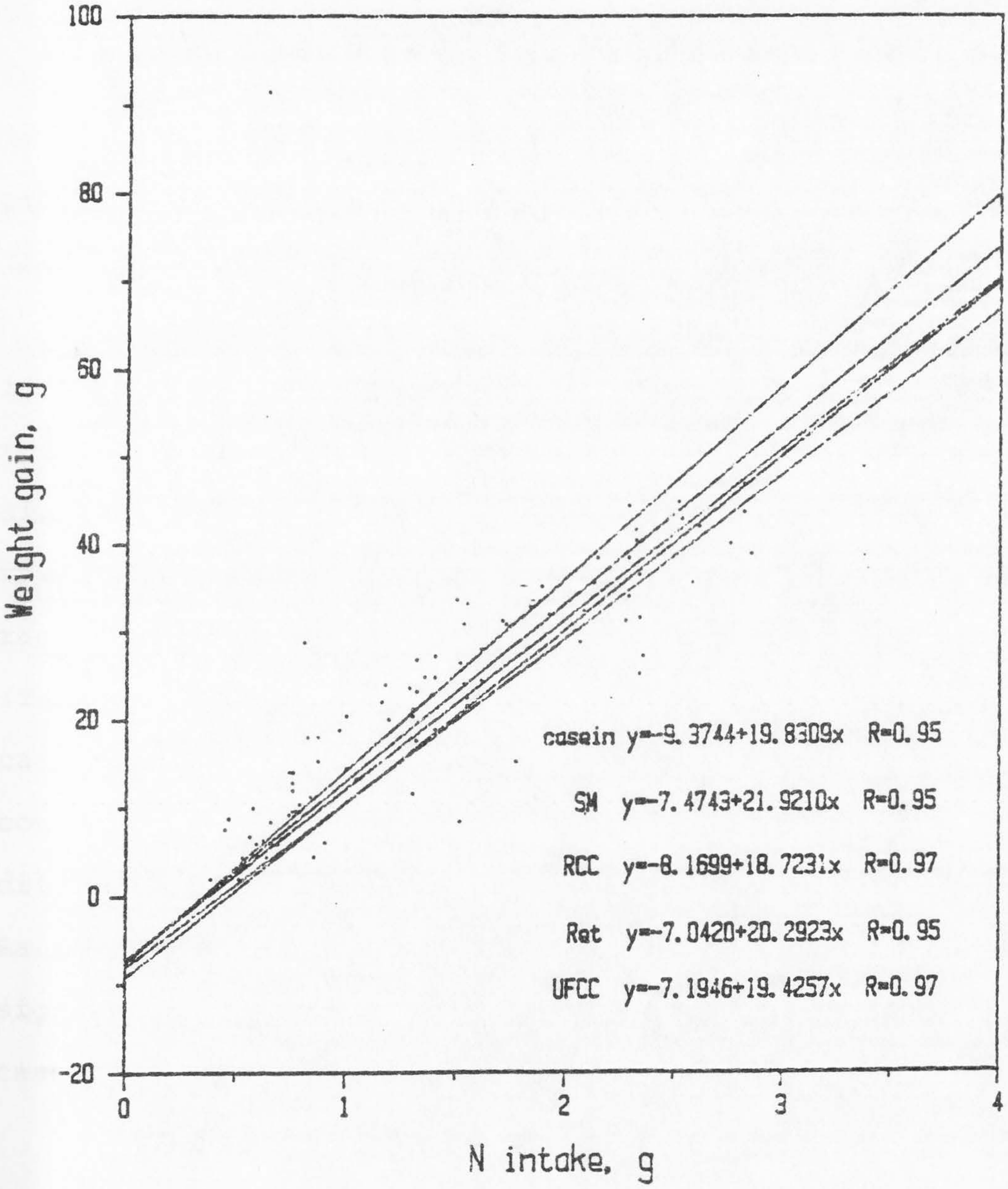

casein y=-0.0598+0.7988x R=0.99
SM y=-0.0796+0.7993x R=0.99
RCC y=-0.0992+0.8322x R=1.00
Ret y=-0.0803+0.8173x R=1.00
UFCC y=-0.0712+0.8136x R=1.00
N balance, g
N intake, g
3
2
1
0
-1
0
1
2
3
4

Table 13. Regression equations of nitrogen intake and nitrogen balance for rats fed different test diets.

Protein	Regression equation	Correlation coefficient	slope	NBI
Ref.casein	y = -0.0598 + 0.7988 x	0.99	0.80	0.80(100)
Skimmilk	y = -0.0796 + 0.7993 x	0.99	0.80	0.80(100)
RCC	y = -0.0992 + 0.8322 x	1.00	0.83	0.83(104)
Retentate	y = -0.0803 + 0.8173 x	1.00	0.82	0.82(103)
UFCC	y = -0.0712 + 0.8136 x	1.00	0.82	0.81(101)

RCC = Regular cottage cheese UFCC = Ultrafiltration cottage cheese
NBI = Nitrogen balance index

Figure 2. Relationship between nitrogen intake and weight gain for rats fed different test diets.


100
80
60
40
20
0
-20
Weight gain, g
0
1
2
3
4
N intake, g
casein y=-9.3744+19.8309x R=0.95
SM y=-7.4743+21.9210x R=0.95
RCC y=-8.1699+18.7231x R=0.97
Rat y=-7.0420+20.2923x R=0.95
UFCC y=-7.1946+19.4257x R=0.97

0.95 for reference casein, 0.91 for skimmilk, 0.97 for regular cottage cheese, 0.95 for retentate and 0.97 for UF cottage cheese. Rats on a non-protein diet lost about 12g during the test period. This represented about 0.2g loss per gram body weight. These rats had a lower food intake (about 60.5g) compared to rats fed the other test diets (Table 14). Food intake increased as percent protein in each sample increased. There was a significant effect ($p<0.001$) of protein level in the diet on mean weight gain of the rats (Appendix F).

At 5% protein level, there was no significant difference in mean weight gain of rats fed the different dietary regimens (Table 14). At 8% protein level, there was also no significant difference in mean weight gain of rats fed the various samples. Mean weight gain at the 8% protein level ranged from 20.5g for reference casein to 25.4g for skimmilk (Table 14). At 11% protein level, rats fed the reference casein diets gained a mean weight of 30.6g, and rats fed UF cottage cheese gained 40.7g (Table 14). The greatest difference in weight gain was observed at the 11% level. Rats fed retentate and UF cottage cheeses gained significantly more weight than rats fed ANRC reference casein, skimmilk, and regular cottage cheese diets.

Regression equations of nitrogen intake on weight gain in the rats is shown in Table 15. From the regression equations the relative nutritive values (RNV) of the test

Table 14. Mean food intake and weight gain for an average of 6 rats fed non-protein and the different diets.

	Protein %	Casein	SM	RCC mean±SE	Ret	UFCC	NP	LSD (0.05)
	NP	--	--	--	--	--	60.5±1.8	--
Initial weight,g	5	61.0±4.4	60.7±3.0	60.7±3.0	60.7±1.7	61.7±2.0	--	NS
	8	60.7±1.8	60.5±1.8	60.8±1.7	60.7±1.8	59.5±2.0	--	NS
	11	60.5±1.7	60.5±1.8	61.5±1.8	61.0±1.7	60.5±1.7	--	NS
LSD		NS	NS	NS	NS	NS	--	--
	NP	--	--	--	--	--	60.4±2.2	--
Food intake, g	5	103.2±7.7	95.4±4.9	97.2±8.3	99.7±1.3	83.2±3.2	--	NS
	8	103.2±2.1	98.4±4.4	98.6±7.2	103.6±5.2	109.6±6.7	--	NS
	11	108.7±2.5	111.3±4.1	121.3±4.1	117.6±4.4	120.1±4.6	--	NS
LSD		NS	NS	NS	NS	NS	--	--
	NP	--	--	--	--	--	-11.9±0.7	--
Weight gain, g	5	7.3±1.4	9.0±1.3	6.8±1.5	12.5±1.0	12.5±7.4	--	NS
	8	20.6±0.8	25.4±1.6	20.7±1.6	23.2±2.7	24.7±1.4	--	NS
	11	30.7±1.3	37.5±1.6	37.9±2.8	40.0±2.4	40.7±2.3	--	7.01
LSD(0.001)		7.06	7.06	7.06	7.06	7.06	--	--

NP = Non-protein group NS = Not significant LSD = Least significant difference
SM = Skimmilk RCC = Regular cottage cheese Ret = Retentate UFCC = Ultrafiltration cottage cheese

Table 15. Regression equations of weight gain nitrogen intake for rats fed the different test diets.

Protein	Regression equation	Correlation coefficient	slope	RNV
Red.casein	y = -9.3744 + 19.8309 x	0.95	19.83	19.83(100)
Skimmilk	y = -7.4743 + 21.9210 x	0.95	21.92	21.92(111)
RCC	y = -8.1699 + 18.7231 x	0.97	18.72	18.72(94)
Retentate	y = -7.0420 + 20.2923 x	0.95	20.29	20.29(102)
UFCC	y = -7.1946 + 19.4257 x	0.97	19.43	19.43(98)

RCC = Regular cottage cheese UFCC = Ultrafiltration cottage cheese
RNV = Relatinve nutritive value

proteins(Hegsted et al.,1968) were calculated to be 111 for skimmilk, 94 for regular cottage cheese, 102 for retentate and 98 for UF cottage cheese. Though the RNV were not significantly different, there was slight improvement for UF cottage cheese compared to regular cottage cheese.

There were significant positive correlations between nitrogen intake (g) and gain in carcass nitrogen (g) of rats fed the different diets. Figure 3 shows that for rats fed ANRC reference casein, the correlation was 0.93. Correlation between nitrogen intake and gain in carcass nitrogen for rats fed skimmilk was 0.95, 0.96 for regular cottage cheese, 0.97 for retentate and 0.99 for UF cottage cheese. Table 16 shows the regression equations between gain in carcass nitrogen and nitrogen intake for rats fed the different diets. The nitrogen growth index (Bodwell, 1977) measures the relationship between gain in carcass nitrogen and nitrogen intake. Algebraically, regression coefficients for nitrogen-growth index (NGI) and nitrogen-balance index (NBI) are similar and a NGI of 0.64 has been reported for rats fed casein diet (Bodwell,1977). NGI values (Table 16) were essentially similar to NBI for each product. NGI values for the products were not significantly different. Compared to reference casein, skimmilk had the highest NGI, followed by retentate. NGI for regular and UF cottage cheese were similar.

Biological value (BV), net protein utilization (NPU) and nitrogen efficiency for growth (NEG) were used for

Figure 3. Relationship between nitrogen intake and gain in carcass nitrogen for rats fed different test diets.

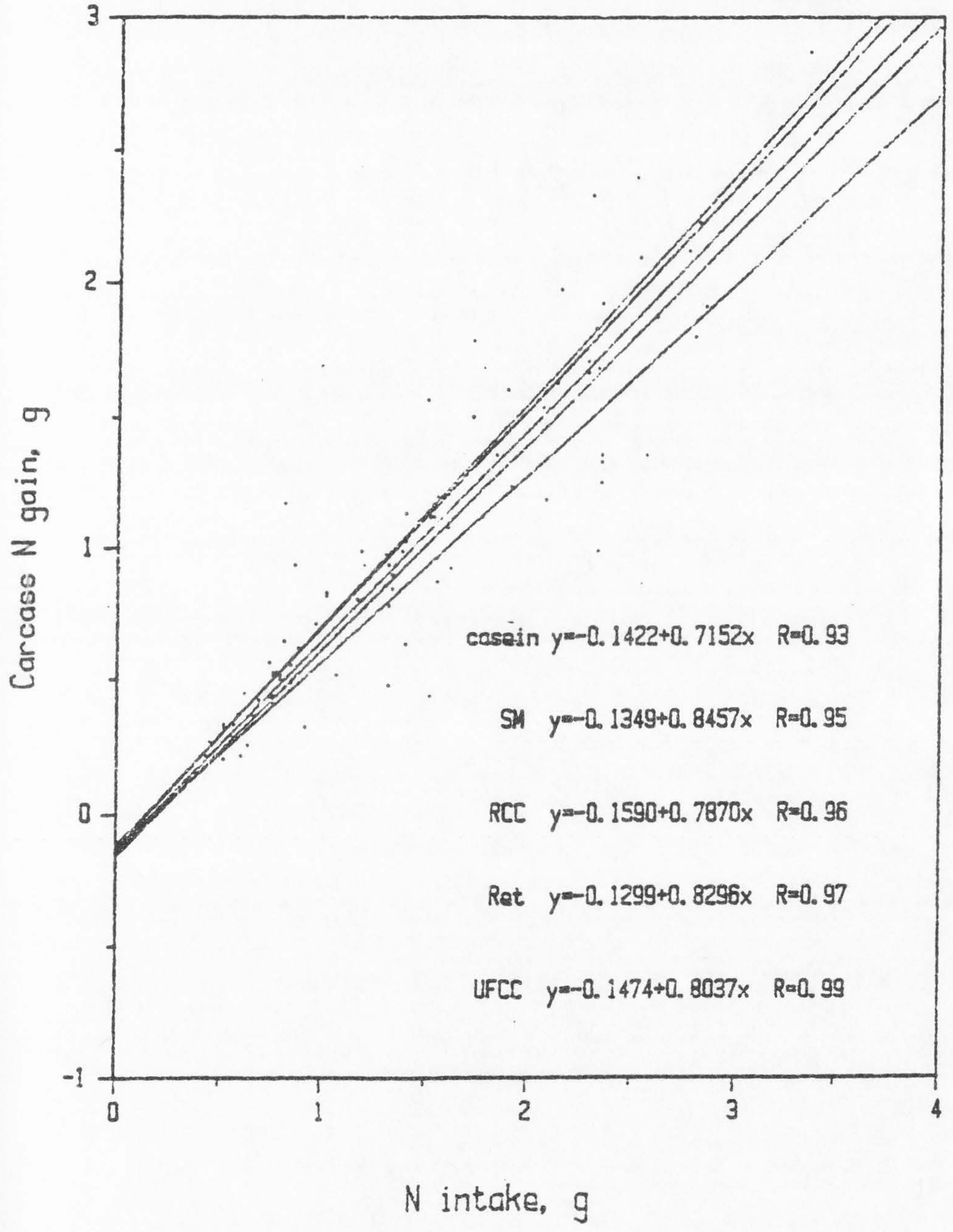

3
2
1
0
-1
Carcass N gain, g
casein y=-0.1422+0.7152x R=0.93
SM y=-0.1349+0.8457x R=0.95
RCC y=-0.1590+0.7870x R=0.96
Ret y=-0.1299+0.8296x R=0.97
UFCC y=-0.1474+0.8037x R=0.99
0
1
2
3
4
N intake, g

Table 16. Regression equations of nitrogen intake and gain in carcass nitrogen for rats fed the different test diets.

Protein	Regression equation	Correlation coefficient	slope	NGI
Red.casein	$y = -0.1422 + 0.7152\ x$	0.93	0.72	0.72(100)
Skimmilk	$y = -0.1349 + 0.8457\ x$	0.95	0.85	0.85(118)
RCC	$y = -0.1590 + 0.7870\ x$	0.96	0.79	0.79(110)
Retentate	$y = -0.1299 + 0.8296\ x$	0.97	0.83	0.83(115)
UFCC	$y = -0.1474 + 0.8037\ x$	0.99	0.80	0.80(111)

RCC = Regular cottage cheese UFCC = Ultrafiltration cottage cheese
NGI = Nitrogen growth index

biological evaluation of the proteins in the test diets. Table 17 shows the BV,NPU and NEG values for the test diets. Average BV was 91±1 for skimmilk, 91±1 for regular cottage cheese, 95±2 for retentate and 94±1 for UF cottage cheese. There was no significant difference ($p<0.05$) in BV for the different diets. Mean NPU ranged from 83±1 in regular cottage cheese to 87±1 for reference casein. NPU values for the test samples were also not statistically different ($p<0.05$). Reference casein and skimmilk had average NEG values of 66±1 and 73±2 respectively. Regular cottage cheese had average NEG values of 70±3, retentate had NEG of 77±1 and the NEG value of UF cottage cheese was 73±1. There was no significant difference($p<0.05$) between NEG values obtained for the different diets.

Dietary proteins lacking in one or more essential amino acids show lower NPU or BV in rats (FAO/WHO ,1973). The FAO/WHO (1973) reported that NPU for cow's milk is 82±4 and that NPU values tend to fall as protein intake increases. NPU values obtained for skimmilk and its products fell within the range reported by FAO/WHO (1973). However, there was no significant effect of protein level in the diet on NPU values ($p<0.05$).

Biological value of milk is lower at 10% protein level than at 5% (Mitchell, 1923b). However, Henry and Don (1957) have suggested using 8% dietary protein level for determination of BV. BV was compared at 5, 8 and 11% protein levels in the diet. There was no significant effect

Table 17. Mean BV, NPU and NEG of reference casein, skimmilk, regular cottage cheese, retentate and ultrafiltration cottage cheese.

Method	Ref.casein	SM	RCC	Ret	UFCC	LSD
			mean ± SE			
BV	93±1	91±1	91±1	95±2	94±1	NS
NPU	87±1	84±2	83±1	85±2	85±1	NS
NEG	66±1	73±2	70±3	77±1	73±1	NS
LSD($p<0.05$)	7.3	7.3	7.3	7.3	7.3	NS

SM = Skimmilk RCC = Regular cottage cheese Ret = Retentate
UFCC = Ultrafiltration cottage cheese LSD = Least significant difference

of percent protein in diet on BV (Appendix G). The FAO (1970) reports that the average BV of casein is 79.7, ranging between 66 and 89. Average BV of the reference casein used in this study was higher than that reported by the FAO.

All three methods (BV,NPU and NEG) differed significantly from each other (Appendix G). BV was the highest and NEG was the lowest for each test sample. The use of NEG as a method of protein evaluation is relatively new and has not gained much acceptance. NEG does not take into account maintenance factors and values obtained for NEG are generally greater as nitrogen intake is above maintenance level (Lopez, 1973). Only two previous studies have reported NEG for casein. Lopez (1973) reported a NEG of 42 for casein and Allred (1976) reported that at 8% protein level, NEG for casein is 65.6. In this study mean NEG for casein was 63, 67 and 67 respectively at 5,8 and 11% protein in the diets. These differences in NEG were not statistically significant at 95% level. There was also no effect of protein level in skimmilk, regular cottage cheese, retentate and UF cottage cheese on NEG values. While NEG values for skimmilk, retentate and UF cottage cheese were slightly higher (Table 17) than that of reference casein and regular cottage cheese, NEG values obtained for the different diets were not significantly different (Appendix E).

A summary of the various biological indexes of the protein quality for the different dairy products is shown in Table 18. From all indications, the protein qualities of the products were not significantly different. However, there was a tendency for a slight improvement of protein quality (about 3%) by ultrafiltration of skimmilk paralleling cheese yield increases of about 2%.

Table 18. A summary of PER, BV, NPU, NEG, NBI, RNV and NGI.

	PER	BV	NPU	NEG	NBI	RNV	NGI
Ref.casein	2.7(2.5)[a]	93(100)	87(100)	66(100)	0.80(100)	20(100)	0.72(100)
Skimmilk	3.0(2.8)	91(98)	84(97)	73(111)	0.80(100)	22(111)	0.85(118)
RCC	2.7(2.5)	91(98)	83(95)	70(106)	0.83(104)	19(94)	0.79(110)
RCC + lactose	2.6(2.4)	------	------	------	--------	------	--------
Retentate	3.1(2.9)	95(102)	85(98)	77(117)	0.82(103)	20(100)	0.83(115)
Retentate + lactose	2.9(2.7)	------	------	------	--------	------	--------
UFCC	2.8(2.6)	94(101)	85(98)	73(111)	0.81(101)	19(98)	0.80(111)
UFCC + lactose	2.7(2.5)	------	------	------	--------	------	---------
LSD	0.19	NS	NS	NS	NS	NS	NS

[a] Values in parenthesis are percentage of ANRC reference casein response.
NS = Not significant. LSD = Least significant difference.
RCC = Regular cottage cheese. UFCC = Ultrafiltration cottage cheese.

CONCLUSIONS

1. The sulfur containing amino acids(cystine + methionine) were the most limiting amino acids in all the products tested. Threonine was the second limiting amino acid in reference casein, skimmilk and regular cottage cheese. There was no second limiting amino acid in retentate and ultrafiltration cottage cheese.

2. There was no significant difference between PER values of reference casein and regular cottage cheese. PER values of retentate was higher than that of casein. Addition of lactose to regular cottage cheese and cottage cheese from retentate significantly lower PER values of these products.

3. Skimmilk, regular cottage cheese, retentate and cottage cheese from ultrafiltrated milk at 5, 8 and 11% protein levels were not significantly different in protein quality measurements due to the protein levels.

4. All 3 methods (BV, NPU and NEG) of measuring protein quality were significantly different from each other. For each sample, BV gave the highest measure and NEG was the lowest. The overall means were 95, 85 and 72% for BV, NPU and NEG respectively.

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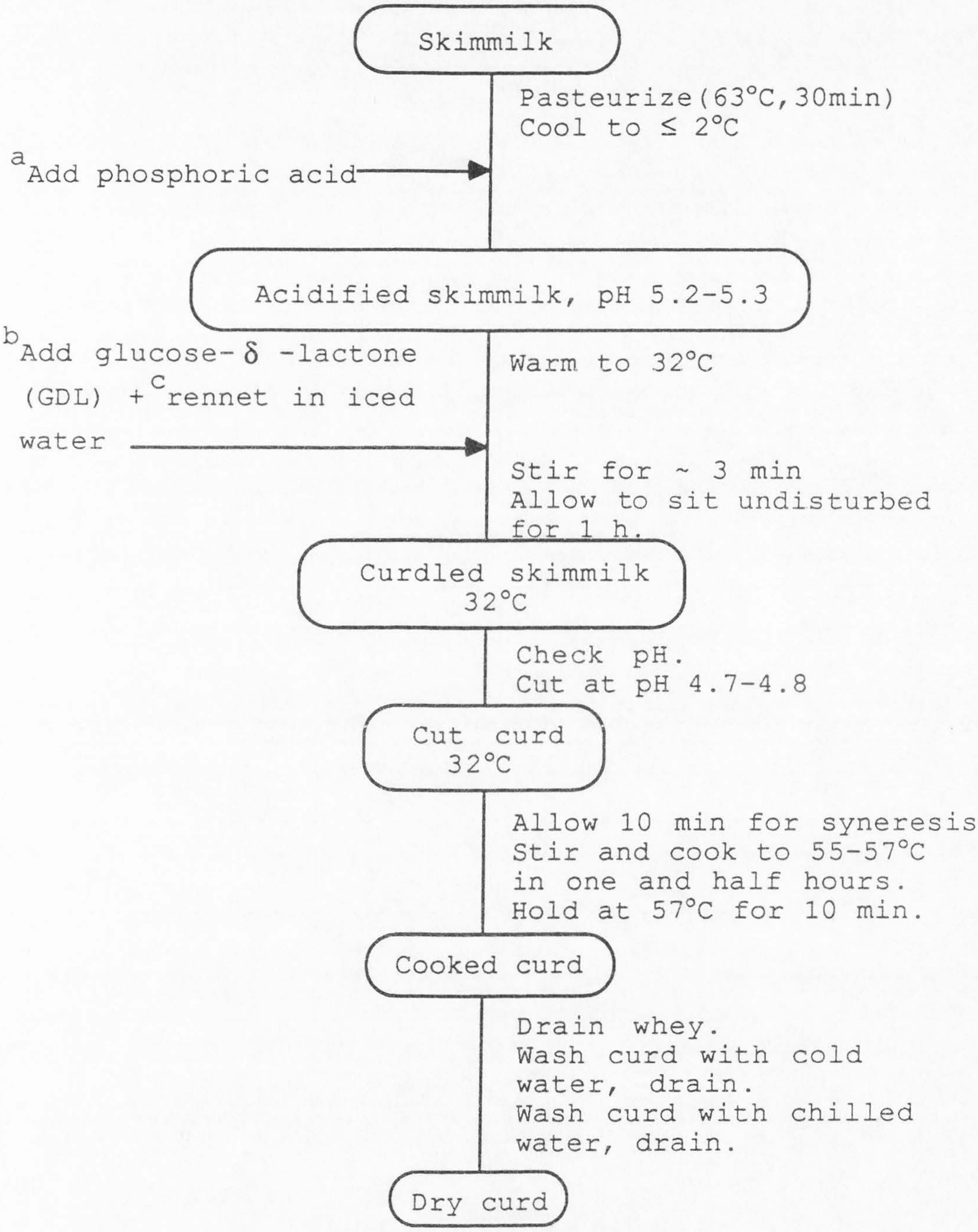
APPENDIXES

Appendix A

(a) Manufacture of Regular Cottage Cheese



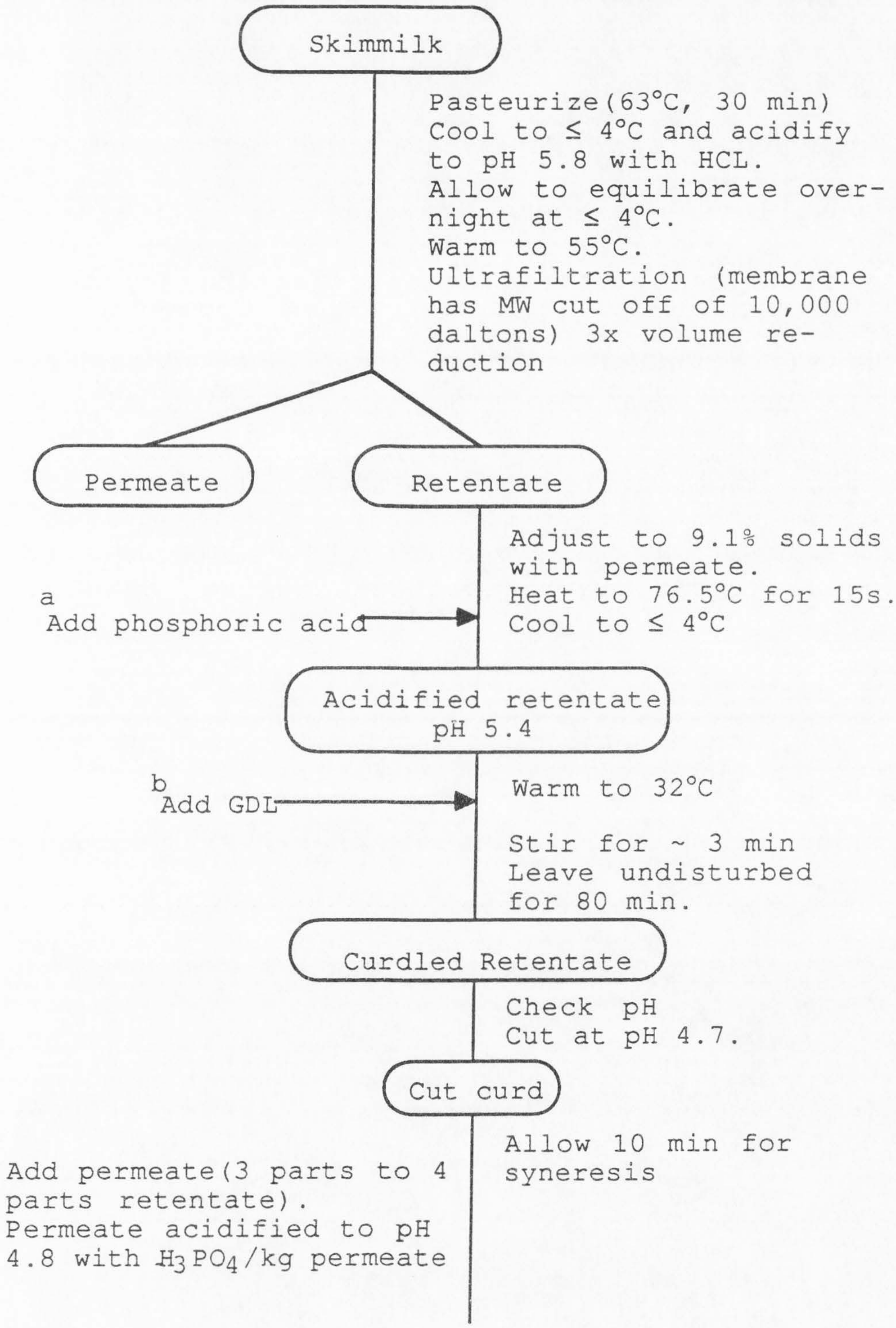
[a] Amount of phosphoric acid (kg) = 0.00893308 x kg milk.

[b] Amount of GDL (kg) = 0.011625462 x kg milk.

[c] Amount of rennet (ml) = 0.006397665 x kg milk.

Appendix A (continue)

(b) Manufacture of UF Cottage Cheese



Appendix A (continue)

Stir & cook curd in permeate t 59°C in 1 h for 50 min. Hold at 59°C for 10 min.

Cooked curd

Drain whey
Wash with iced water and drain.

Dry curd

[a]Amount of phosphoric acid (kg) = 0.00893308 x kg milk.
[b]Amount of GDL (kg) = 0.011625462 x kg milk..

Appendix B

Sample Calculations for Formulation of Diet

Sample calculation for skimmilk

Protein source:

$$\frac{\%\ \text{protein desired} \times \text{total wt.(g)}}{\%\ \text{in sample}} = \frac{0.05 \times 1300(g)}{0.36} = 180.58g$$

Mineral mix:

g mineral desired - (protein source (g) x % ash in sample)

0.05 x 1300 g - (180.57 x .078)= 50.96 g

Corn oil:

g corn oil desired - (protein source g x % fat in sample)

= 0.08 x 1300 g - (180.57 x .01) = 102.18 g

Vitamins:

% desired x total wt. = .01 x 1300g = 13.0 g

Cellulose:

% desired x total wt. = .01 x 1300g = 13.0 g

Glucose:

1300 g - (protein source + minerals + oil + vitamins + cellulose).

= 1300g - 270.53 g = 1029.47g

Appendix C

Composition of Vitamin Supplements

Composition	g/kg
Alpha Tocopherol	5.0
L Ascorbic Acid	45.0
Choline Chloride	75.0
D-Calcium Pantothenate	3.0
Inositol	5.0
Menadione	2.25
Niacin	4.5
PABA	5.0
Pyridoxine HCL	1.0
Riboflavin	1.0
Thiamin HCL	1.0
Vitamin A Acetate	0.31
Calciferol (D_2)	0.003
	mg/kg
Biotin	20.0
Folic Acid	90.0
Vitamin B_{12}	1.4

Appendix D

Mineral Composition

Composition mixture	g/kg
Calcium phosphate, dibasic ($CaHPO_4$)	500.0
Sodium chloride (NaCl)	74.0
Potassium citrate monohydrate ($(CH_2COOK)_2H_2O$)	220.0
Potassium sulfate (K_2SO_4)	52.0
Magnesium oxide (MgO)	24.0
Manganous carbonate (42-48% mm)	3.5
Ferric citrate (16-17% Fe)	6.0
Zinc carbonate (70% ZnO)	1.6
Cupric carbonate (53-55% Cu)	0.3
Potassium iodate (KLO_3)	0.01
Sodium selenite ($Na_2SeO_35H_2O$)	0.01
Chromium potassium sulfate $CrK(SO_4)_2\ 12H_2O$	0.55
Sucrose, finely powdered	118.00

Appendix E

Analysis of Variance Tables at 10% Protein Level

a) Analysis of variance on PER

SV	df	SS	MS	F
Treatment	7	1.948	0.278	5.90**
Error	72	3.394	0.047	
Total	79	5.343		

**Significant at $p < 0.001$

b) Analysis of variance on weight gain

SV	df	SS	MS	F
Treatment	7	4842	692	5.99**
Error	72	8310	115	
Total	79	13152		

**Significant at $p < 0.001$

c) Analysis of variance on protein intake

SV	df	SS	MS	F
Treatment	7	237.0	33.9	2.87*
Error	72	850.6	11.8	
Total	79	1087.6		

*Significant at $p < 0.05$

Appendix F

Analysis of Variance on Weight Gain of Rats Fed Diets at 5, 8 and 11 % Protein Levels

SV	df	SS	MS	F
Diet	4	499.502	124.876	3.202*
Level	2	11538.932	5769.466	147.957**
Diet x Level	8	182.687	22.836	<1.000
Error	75	2924.565	38.994	
Total	89	15145.686	170.176	

* Significant at $p < 0.05$
**Significant at $p < 0.001$.

Appendix G

Analysis of Variance on Diet, Protein Level and Method

SV	df	SS	MS	F
Diet	4	1417.023	354.255	<1
Level	2	23.705	11.852	<1
Diet x level	8	1275.509	159.938	<1
Error H	75	30791.050	410.547	
Method	2	7308.669	3654.334	7.581*
Diet x method	8	5678.768	703.596	1.460
Level x method	4	2937.169	734.292	1.523
Diet x level x method	16	3770.149	235.634	<1
Error R	150	72301.625	482.010	

* Significant at $p < 0.05$

Appendix H

Mean BV, NPU and NEG for reference casein, skimmilk, regular cottage cheese, retentate and UF cottage cheese of rats fed at 5, 8 and 11 % protein levels.

	Ref.casein			SM			RCC			Ret			UFCC		
Protein level %	BV	NPU	NEG	BV	NPU	NEG	BV	NPU	NEG	BV	NPU	NEG	BV	NPU	NEG
5	95	89	63	89	79	68	92	84	64	99	90	74	95	84	70
8	92	86	67	93	87	76	89	81	74	94	82	76	95	88	73
11	92	85	67	91	85	74	91	84	73	93	84	82	92	83	75
$\bar{X}$ (overall)	93	87	66	91	84	73	91	83	70	95	85	77	94	85	73
S E	1	1	1	1	2	2	1	1	3	2	2	1	1	1	1

Each mean represents six observations.

SM = Skimmilk RCC = Regular cottage cheese Ret = Retentate
UFCC = Ultrafltration cottage cheese

Appendix I. Raw Data on Individual Rats

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Rat #	Trtmnt	INIT BW	FIN BW	Food Int	Food P	food N	FECAL N	URINE N	CARCA N	INT C N	WT GAIN	NEG	PER	NPU	BV
2	44	51	58	66.51	91.38	4.57	.716	.14	.12	1.62	1.22	8.51	69.76	1.86	81.39	89.28
3	45	51	62	68.8	84.56	4.23	.663	.18	.19	1.61	1.30	6.8	61.23	1.61	66.32	75.65
4	54	51	62	66.29	90.43	4.52	.708	.14	.19	1.59	1.30	4.29	50.66	.95	71.30	78.30
5	17	51	55	64.96	86.18	4.31	.676	.17	.08	1.6	1.16	9.96	88.02	2.31	84.71	98.28
6	25	51	58	71.02	116.02	5.8	.909	.18	.06	1.74	1.22	13.02	71.60	2.24	87.54	98.76
7	31	51	69	80.26	103.82	5.19	.813	.2	.08	1.9	1.45	11.26	73.52	2.17	81.16	95.65
8											0.0		Error			
9	AVE		60.67	69.64	95.40	4.77	.75	.17	.12	1.68	1.27	8.97	69.11	1.86	78.74	89.35
10	SUM		364.00	417.84	572.39	28.62	4.49	.99	.70	10.06	7.64	53.84	414.78	11.14	472.42	536.13
11	SUMSQU		22202.00	29256.69	55344.51	138.38	3.40	.17	.10	16.94	9.79	532.57	29460.34	22.04	37531.50	48417.43
12	STDEV		4.89	5.63	12.16	.61	.10	.02	.06	.12	.10	3.14	12.54	.52	8.19	10.12
13																
14																
15	30	52	55	57.19	81.52	4.076	.639	.09	.11	1.5	1.16	2.19	62.66	.54	88.53	90.41
16	88	52	58	63.88	107.53	5.38	.843	.14	.09	1.57	1.22	5.88	50.05	1.09	87.75	94.87
17	92	52	59	67.58	137.52	6.88	1.078	.14	.08	1.69	1.24	8.58	48.06	1.25	91.35	97.04
18	2	52	62	73.9	98.75	4.94	.774	.12	.08	1.78	1.30	11.9	73.06	2.41	90.53	95.90
19	23	52	63	68.8	94.4	4.72	.740	.14	.06	1.78	1.32	5.8	78.19	1.23	90.09	98.52
20	27	52	69	78.68	99.71	4.98	.781	.16	.1	1.88	1.45	9.68	69.45	1.94	82.92	92.83
21											0.0		Error			
22	AVE		61.00	68.34	103.24	5.16	.81	.13	.09	1.70	1.28	7.34	63.28	1.41	88.53	94.93
23	SUM		366.00	410.03	619.43	30.98	4.86	.79	.52	10.20	7.69	44.03	379.67	8.46	531.17	569.57
24	SUMSQR		22444.00	28303.60	65724.97	164.37	4.04	.11	.05	17.44	9.90	381.94	24731.99	14.13	47070.51	54111.84
25	STDEV		4.86	7.52	18.85	.94	.15	.02	.02	.14	.10	3.43	11.89	.66	3.05	2.94
26																
27																
28											0.0		Error			
29	56	53	58	73.21	105.77	5.29	.829	.07	.08	1.78	1.22	15.21	74.03	2.88	97.19	96.41
30	66	53	68	77.58	100.73	5.04	.790	.21	.04	1.9	1.43	9.56	81.38	1.90	84.39	101.52
31	99	53	62	71.51	97.96	4.9	.768	.2	.07	1.7	1.30	9.51	70.07	1.94	81.34	96.00
32	3	53	59	71.72	98.02	4.9	.768	.11	.02	1.62	1.24	12.72	57.90	2.60	99.57	104.08
33	32	53	55	68.8	97.95	4.9	.768	.14	.06	1.68	1.16	13.8	83.60	2.82	90.46	98.58
34	42	53	63	77.07	97.79	4.89	.766	.15	.08	1.81	1.32	14.07	79.00	2.88	86.52	95.67
35																
36	AVE		60.83	73.31	99.70	4.99	.78	.15	.06	1.75	1.28	12.48	74.33	2.50	89.91	98.86
37	SUM		365.00	439.89	598.22	29.92	4.69	.88	.35	10.49	7.67	74.89	445.98	15.01	539.47	593.17
38	SUMSQR		22307.00	32309.02	59694.99	149.33	3.67	.14	.02	18.39	9.84	963.76	33594.77	38.60	48767.01	58695.79
39	STDEV		4.54	3.42	3.18	.16	.02	.05	.02	.10	.10	2.41	9.44	.46	7.24	3.30
40																
41																
42																
43	77	54	62	72.47	100.4	5.02	.787	.14	.12	1.8	1.30	10.47	76.99	2.09	83.06	90.33
44	84	54	63	68.91	87.72	4.39	.688	.12	.13	1.52	1.32	5.91	34.68	1.35	82.08	87.59
45	101	54	59	61.08	70.81	3.54	.555	.19	.11	1.36	1.24	2.08	33.16	.59	68.77	86.41
46	36	54	55	66.5	130.91	6.55	1.027	.11	.08	1.7	1.16	11.5	59.46	1.76	93.83	96.98
47	38	54	67	73.43	89.23	4.46	.699	.17	.09	1.85	1.41	6.43	83.73	1.44	80.93	93.40
48	71	54	58	82.27	104.23	5.21	.817	.13	.05	1.87	1.22	4.27	94.96	.82	93.47	100.00
49											0.0					
50	AVE		60.67	67.44	97.22	4.86	.76	.14	.10	1.68	1.27	6.78	63.83	1.34	83.69	92.45
51	SUM		364.00	404.66	583.30	29.17	4.57	.86	.58	10.10	7.64	40.66	382.98	8.04	502.15	554.71

Appendix I (continue)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
52	SUMSQR		22172.00	27423.02	58752.33	146.94	3.61	.13	.06	17.21	9.78	340.70	27793.34	12.34	42456.87	51426.20
53	STDEV		4.23	5.13	20.23	1.01	.16	.03	.03	.20	.09	3.61	25.88	.56	9.29	5.35
54																
55																
56													Error			
57	73	55	55	62.73	75.45	3.77	.591	.12	.1	1.37	1.16	7.73	45.66	2.05	84.21	90.87
58	80	55	57	66.38	97.18	4.86	.762	.15	.06	1.72	1.20	9.38	85.40	1.93	89.06	98.55
59	85	55	62	66.59	81.9	4.1	.643	.16	.09	1.7	1.30	4.59	82.46	1.12	80.81	92.85
60	14	55	63	67.86	85.7	4.28	.671	.13	.07	1.7	1.32	4.86	69.71	1.14	89.07	96.76
61	37	55	67	70.78	82.13	4.11	.644	.1	.09	1.76	1.41	3.78	64.87	.92	90.17	93.56
62	72	55	66	74.75	78.96	3.85	.603	.24	.07	1.65	1.39	8.75	72.64	2.27	69.62	95.46
63											0.0					
64	AVE		61.67	68.18	83.22	4.16	.65	.15	.08	1.65	1.30	6.51	70.14	1.57	83.83	94.67
65	SUM		370.00	409.09	499.32	24.97	3.91	.90	.48	9.90	7.77	39.09	420.82	9.43	502.96	568.04
66	SUMSQR		22932.00	27977.94	41856.03	104.68	2.57	.15	.04	16.44	10.11	283.28	30536.34	16.48	42467.77	53817.40
67	STDEV		4.80	4.14	7.79	.39	.08	.05	.02	.14	.10	2.39	14.29	.58	7.83	2.79
68																
69																
70											0.0		Error			
71	0	0	55	45.12	56.36			.04	.02	1.04	1.16	-0.88	287.50	-Infinit	Infinity	Infinity
72	53	0	63	48.65	67.98			.09	.06	1.31	1.32	-14.35	14.44	-Infinit	-Infinit	-Infinit
73	58	0	60	49.67	67.58			.06	.05	1.32	1.26	-10.33	-100.00	-Infinit	Infinity	Infinity
74	59	0	67	55.2	60.63			.11	.05	1.51	1.41	-11.8	-93.64	-Infinit	-Infinit	-Infinit
75	96	0	61	48.36	57.68			.06	.07	1.12	1.28	-12.64	268.33	-Infinit	-Infinit	-Infinit
76	97	0	57	44.48	52.62			.09	.08	1.16	1.20	-12.52	41.11	-Infinit	-Infinit	-Infinit
77	AVE															
78	SUM		306.00	247.00	310.23	0.0	0.0	.36	.25	6.30	6.43	-59.00	Error	-Infinit	Error	Error
79	SUMSQR		18804.00	12255.48	19367.77	0.0	0.0	.03	.01	8.07	8.29	709.26	Error	Infinity	Infinity	Infinity
80	STDEV		4.38	3.66	5.46	0.0	0.0	.03	.02	.18	.53	1.81	Error	Error	Error	Error
81																
82																
83											0.0		Error			
84											0.0		Error			
85	64	81	61	81.21	91.58	7.33	1.149	.13	.21	2.03	1.28	20.21	73.51	2.76	81.43	85.40
86	68	81	57	87.76	109.6	8.77	1.375	.25	.13	2.08	1.20	30.76	78.52	3.51	81.57	93.34
87	89	81	55	81.62	102.08	8.17	1.281	.19	.16	1.91	1.16	26.62	60.23	3.26	82.56	90.58
88	4	81	67	95.7	80.54	6.44	1.009	.11	.07	2.2	1.41	28.7	88.17	4.46	94.72	97.95
89	8	81	60	83.47	99.8	7.98	1.251	.15	.09	2.09	1.26	23.47	75.40	2.94	90.94	96.60
90	63	81	63	85.84	106.97	8.56	1.342	.13	.13	2.15	1.32	22.84	68.25	2.67	90.06	93.79
91											0.0		Error			
92	AVE		60.50	85.93	98.43	7.88	1.23	.16	.13	2.08	1.27	25.43	75.51	3.26	86.88	92.94
93	SUM		363.00	515.60	590.57	47.25	7.41	.96	.79	12.46	7.62	152.60	453.08	19.59	521.30	557.66
94	SUMSQR		22053.00	44452.94	58708.70	375.82	9.23	.17	.12	25.93	9.73	3959.44	34478.83	66.15	45456.13	51932.26
95	STDEV		4.28	5.40	10.77	.86	.14	.05	.05	.10	.09	3.96	7.29	.66	5.74	4.51
96																
97																
98											0.0		Error			
99	1	82	67	88.7	110.98	8.88	1.392	.12	.1	2.42	1.41	21.7	79.65	2.44	93.30	98.29
100	6	82	60	78.84	107.52	8.6	1.348	.13	.11	1.84	1.28	18.84	47.62	2.19	91.50	95.37
101	16	82	64	83.95	99.68	7.97	1.249	.19	.11	2.15	1.34	18.95	76.09	2.50	88.13	94.72
102	48	82	57	80.08	101.94	8.15	1.277	.18	.18	1.91	1.20	23.08	84.97	2.83	83.30	90.63

Appendix I (continue)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
103	90	82	55	77.19	97.31	7.78	1.219	.14	.18	1.91	1.16	22.19	60.94	2.85	84.15	88.76
104	102	82	61	78.74	101.5	8.12	1.273	.17	.21	1.97	1.28	17.74	62.48	2.18	80.10	86.43
105											0.0		Error			
106	AVE		60.67	81.25	103.16	8.25	1.29	.15	.14	2.03	1.27	20.58	66.72	2.50	86.43	92.03
107	SUM		364.00	487.50	618.93	49.50	7.76	.93	.87	12.20	7.64	123.50	400.78	15.01	518.57	552.20
108	SUMSQR		22180.00	39702.13	63976.46	409.22	10.05	.15	.14	25.04	9.78	2563.63	27418.97	37.96	44947.69	50900.75
109	STDEV		4.41	4.31	5.11	.41	.06	.03	.05	.22	.09	2.08	11.41	.29	5.08	4.01
110																
111																
112											0.0		Error			
113	61	83	61	82.24	100.69	8.08	1.263	.22	.16	2.1	1.28	21.24	78.50	2.64	79.95	90.18
114	81	83	60	87.13	107.54	8.6	1.348	.28	.07	2.10	1.26	27.13	87.08	3.15	83.43	98.25
115	104	83	55	79.66	103.77	8.3	1.301	.17	.14	1.96	1.16	24.66	71.18	2.97	85.91	92.55
116	21	83	64	82.02	81.52	6.52	1.022	.31	.17	1.8	1.34	18.02	64.05	2.76	65.43	84.78
117	41	83	67	82	117.82	9.42	1.476	.17	.09	2.5	1.41	15	83.66	1.59	90.97	97.11
118	60	83	57	90.22	110.27	8.82	1.382	.23	.05	1.99	1.20	33.22	68.81	3.77	88.91	100.00
119											0.0		Error			
120	AVE		60.67	83.88	103.60	8.29	1.30	.23	.11	2.09	1.27	23.21	75.55	2.81	82.43	93.81
121	SUM		364.00	503.27	621.61	49.72	7.79	1.38	.68	12.54	7.64	139.27	453.28	16.88	494.61	562.87
122	SUMSQR		22180.00	42291.70	65158.08	416.85	10.24	.33	.09	26.50	9.78	3448.58	34648.03	50.08	41195.73	52969.16
123	STDDEV		4.41	3.96	12.31	.98	.15	.06	.05	.24	.09	6.57	8.99	.72	9.20	5.75
124																
125																
126											0.0		Error			
127	40	84	56	76.44	100.29	8.02	1.257	.13	.26	2	1.18	20.44	73.11	2.55	79.05	82.55
128	87	84	64	78.49	91.48	7.32	1.147	.17	.21	2.06	1.34	14.49	73.26	1.98	77.92	84.82
129	100	84	61	85.25	114.86	9.19	1.440	.2	.19	2.2	1.28	24.25	74.09	2.64	81.72	89.37
130	26	84	57	81.72	107.54	8.6	1.348	.23	.11	2.15	1.20	24.72	85.24	2.87	84.18	94.96
131	28	84	60	82.42	110.36	8.83	1.384	.16	.09	1.99	1.26	22.42	59.64	2.54	91.09	96.92
132	34	84	67	85.01	66.74	8.34	1.307	.27	.22	2.2	1.41	18.01	76.46	2.16	72.21	84.74
133											0.0		Error			
134	AVE		60.83	81.56	98.55	8.38	1.31	.19	.18	2.10	1.28	20.72	73.63	2.46	81.03	88.90
135	SUM		365.00	489.33	591.27	50.30	7.86	1.16	1.08	12.60	7.67	124.33	441.80	14.74	486.17	533.39
136	SUMSQR		22291.00	39969.23	59817.90	423.84	10.41	.24	.22	26.51	9.83	2653.91	32870.05	36.75	39597.02	47592.66
137	STDEV		4.17	3.52	17.61	.66	.10	.05	.07	.10	.09	3.94	8.24	.33	6.37	5.93
138																
139																
140											0.0		Error			
141	67	85	60	83.9	98.65	7.89	1.237	.15	.16	2.1	1.26	23.9	77.30	3.03	85.18	90.54
142	78	85	64	91.14	138.2	11.06	1.734	.31	.12	2.39	1.34	27.14	73.48	2.45	82.50	95.33
143	103	85	54	72.61	87.33	6.99	1.096	.16	.12	1.76	1.13	18.61	66.91	2.66	86.01	93.09
144	7	85	56	79.94	109.47	8.76	1.373	.11	.11	2.3	1.18	23.94	89.99	2.73	93.20	95.52
145	19	85	67	93.37	108.53	8.68	1.361	.18	.12	2.23	1.41	26.37	69.72	3.04	87.26	94.43
146	43	85	56	83.93	115.46	9.24	1.448	.16	.07	1.95	1.18	27.93	60.08	3.02	92.87	98.53
147											0.0		Error			
148	AVE		59.50	84.15	109.61	8.77	1.37	.18	.12	2.12	1.25	24.65	72.75	2.82	87.84	94.58
149	SUM		357.00	504.89	657.64	52.62	8.25	1.07	.70	12.73	7.50	147.89	436.47	16.94	527.02	567.45
150	SUMSQR		21373.00	42770.53	73551.04	470.89	11.57	.21	.09	27.29	9.43	3702.71	32240.57	48.12	46385.72	53702.66
151	STDEV		5.13	7.55	17.14	1.37	.22	.07	.03	.24	.11	3.59	9.66	.24	4.32	2.67
152																
153																

Appendix I (continue)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
154											0.0		Error			
155	88	111	60	95.3	105.15	11.57	1.813	.18	.21	2.5	1.26	35.3	75.91	3.05	85.48	90.64
156	95	111	65	101.4	117.22	12.89	2.020	.23	.3	2.8	1.36	38.4	80.15	2.82	80.04	88.61
157	105	111	60	96.48	117.15	13	2.038	.33	.27	2.09	1.26	36.48	48.61	2.81	76.77	87.67
158	11	111	56	89.49	93.94	10.33	1.619	.12	.18	2.5	1.18	33.49	88.32	3.24	90.53	93.02
159	51	111	66	110.74	120.08	13.21	2.071	.25	.19	3	1.39	44.74	88.66	3.39	84.87	92.62
160	52	111	56	94.30	114.5	12.6	1.975	.17	.18	2.26	1.18	38.30	60.08	3.05	80.71	94.15
161											0.0		Error			
162	AVE		60.50	97.97	111.34	12.27	1.92	.21	.21	2.52	1.27	37.47	73.62	3.06	84.57	90.79
163	SUM		363.00	587.80	668.04	73.60	11.54	1.28	1.29	15.15	7.62	224.80	441.70	18.36	507.40	544.72
164	SUMSQR		22053.00	57853.72	74875.35	908.99	22.33	.30	.29	38.82	9.73	8498.88	33816.09	56.42	43052.92	49500.00
165	STDEV		4.28	7.33	9.96	1.11	.17	.07	.08	.34	.09	3.91	16.12	.23	5.37	3.06
166																
167																
168											0.0		Error			
169	5	112	56	86.68	101.07	11.12	1.743	.18	.18	2.5	1.18	30.68	83.64	2.78	88.91	93.37
170	49	112	66	99.83	114.31	12.57	1.970	.23	.33	2.8	1.39	33.83	69.76	2.69	78.01	84.59
171	35	112	65	90.28	116.54	12.82	2.009	.19	.27	2.1	1.36	25.28	40.40	1.97	83.41	88.40
172	62	112	56	88.29	109.4	12.03	1.886	.28	.11	2.08	1.18	32.29	55.61	2.68	87.10	96.48
173	10	112	60	88.97	108.52	11.94	1.871	.32	.16	2.6	1.26	28.97	86.37	2.43	81.12	93.24
174	18	112	60	92.83	102.22	11.24	1.762	.17	.16	2.32	1.26	32.83	66.59	2.92	88.46	93.41
175											0.0		Error			
176	AVE		60.50	91.15	108.68	11.95	1.87	.22	.20	2.37	1.27	30.65	67.06	2.58	84.50	91.58
177	SUM		363.00	546.88	652.06	71.72	11.24	1.33	1.19	14.20	7.62	183.88	402.37	15.45	507.01	549.49
178	SUMSQR		22053.00	49958.12	71057.37	859.63	21.12	.31	.27	33.89	9.73	5684.52	28481.44	40.37	42939.66	50414.58
179	STDEV		4.28	4.73	6.22	.68	.11	.06	.08	.24	.09	3.14	17.30	.34	4.40	4.30
180																
181																
182											0.0		Error			
183	74	113	60	102.72	120.57	13.26	2.078	.18	.31	2.8	1.26	42.72	81.12	3.22	82.52	86.84
184	94	113	66	104.91	129.96	14.3	2.241	.27	.3	2.72	1.39	38.91	67.67	2.72	80.22	87.70
185	83	113	56	91.76	111.87	12.31	1.929	.35	.17	2.5	1.18	35.76	83.83	2.90	79.62	92.75
186	13	113	65	96.18	100.86	11.09	1.738	.23	.09	2.77	1.36	31.18	93.15	2.81	88.88	97.48
187	69	113	59.8	107.08	127.57	14.03	2.199	.23	.19	2.4	1.26	47.28	58.11	3.37	86.66	93.16
188	24	113	56	100.35	114.74	12.62	1.978	.29	.1	3	1.18	44.35	108.05	3.51	86.69	97.17
189											0.0		Error			
190	AVE		60.47	100.50	117.59	12.94	2.03	.28	.19	2.70	1.27	40.03	81.99	3.09	84.10	92.53
191	SUM		362.80	603.00	705.57	77.61	12.16	1.55	1.16	16.19	7.62	240.20	491.93	18.54	504.59	555.18
192	SUMSQR		22029.04	60764.25	83553.74	1010.95	24.84	.42	.27	43.92	9.71	9792.28	41016.53	57.83	42508.78	51472.68
193	STDEV		4.28	5.71	10.79	1.19	.19	.06	.09	.22	.09	5.94	17.80	.32	3.84	4.50
194																
195																
196											0.0		Error			
197	12	114	66	114.75	140.18	15.42	2.417	.21	.22	3.27	1.39	48.75	85.37	3.16	87.45	92.56
198	20	114	60	98.37	112.55	12.38	1.940	.19	.17	2.8	1.26	38.37	87.98	3.10	87.98	93.43
199	22	114	60	91.46	117.11	12.88	2.019	.11	.18	2.27	1.26	31.46	52.91	2.44	91.91	93.45
200	29	114	56	98.13	124.61	13.71	2.149	.38	.23	2.6	1.18	42.13	79.60	3.07	78.44	90.35
201	82	114	58	93.42	122.54	13.48	2.113	.21	.29	2.2	1.22	35.42	51.61	2.63	82.33	87.88
202	57	114	56	97.36	114.02	12.54	1.968	.35	.2	2.7	1.39	31.36	81.34	2.50	78.46	91.14
203											0.0		Error			
204	AVE		61.00	98.91	121.84	13.40	2.10	.24	.21	2.64	1.28	37.91	73.13	2.82	84.43	91.47

Appendix I (continue)

	A	B	C	D	E	F	G	H	I
205	SUM		366.00	593.49	731.01	80.41	12.60	1.43	1.29
206	SUMSQR		22412.00	59044.91	89576.95	1083.86	26.63	.39	.29
207	STDEV		4.15	8.24	10.14	1.12	.18	.10	.04
208									
209									
210									
211	76	115	56	97.79	116.48	12.8	2.006	.21	.32
212	93	115	60	91.84	106.33	11.7	1.834	.15	.26
213	79	115	56	96.3	115.76	12.73	1.995	.22	.25
214	48	115	60	100.02	125.45	13.8	2.163	.21	.2
215	15	115	66	112.62	128.46	14.13	2.215	.36	.13
216	39	115	66	109.68	128.06	14.09	2.208	.48	.07
217									
218	AVE		60.67	101.38	120.09	13.21	2.07	.27	.21
219	SUM		364.00	608.25	720.54	79.25	12.42	1.63	1.23
220	SUMSQR		22184.00	61988.13	86913.07	1051.41	25.83	.52	.29
221	STDEV		4.50	8.08	8.76	.96	.15	.12	.09
222									
223									
224									
225									
226		skim=1							
227		anrc=2							
228		ret=3							
229		cc=4							
230		uf=5							

	J	K	L	M	N	O	P
205	15.84	7.69	227.49	438.80	16.90	506.57	548.80
206	42.58	9.88	8851.51	33442.76	48.17	42923.23	50220.91
207	.39	.09	6.73	16.44	.33	5.54	2.16
208							
209							
210		0.0		Error			
211	2.4	1.18	41.79	68.14	3.26	79.90	85.58
212	2.21	1.26	31.84	56.42	2.72	84.55	88.07
213	2.6	1.18	40.3	80.21	3.17	82.79	89.20
214	2.9	1.26	40.02	83.97	2.90	86.90	92.61
215	2.8	1.39	46.62	76.24	3.30	83.60	95.86
216	2.85	1.39	43.68	84.70	3.10	80.83	98.89
217		0.0		Error			
218	2.63	1.27	40.71	74.95	3.08	83.10	91.70
219	15.76	7.64	244.25	449.68	18.45	498.58	550.22
220	41.78	9.78	10067.25	34297.78	56.99	41461.94	50583.12
221	.28	.09	4.98	10.91	.22	2.54	5.03
222							
223							
224							
225							
226							
227							
228							
229							
230							