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Protein Nutritional Quality of Florunner Peanut Meal as Measured by Rat Bioassay

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Maximum growth rate of weanling rats was obtained with diets containing peanut meal as the sole source of dietary protein. The meal was made from blanched peanuts of the Florunner variety by cold expression and solvent extraction of the oil. All post-harvest treatment and handling conditions were selected to minimize changes in protein quality. Growth of rats fed 16.7 and 20% peanut protein was essentially equivalent to that of animals fed 12 to 24% casein protein. With 13.3% peanut protein in the diet, methionine, lysine, and threonine were equally limiting in the peanut meal as measured by rat growth and protein efficiency ratio (PER) of amino acid supplemented diets.

Peanuts are grown as an oilseed crop in many developing countries of the world. The defatted residue, or peanut cake, is an abundant and inexpensive source of protein readily available in some areas where protein deficiency is widespread. Unsanitary practices in many of the oil mills currently preclude use of the peanut cake for human or animal consumption, but such practices could be altered. Most of the peanuts produced in the United States are marketed for food uses as full-fat products. Some nuts are crushed for oil, however, and this might become a more significant outlet for the crop if production regulations were altered. Thus, an economical utilization of the residue remaining after oil extraction from peanuts might be beneficial both financially and nutritionally.

The protein of peanut meal is considered to be of low quality because several of the essential amino acids are present in low proportions compared with total protein. Estimates of the biological value of peanut protein relative to that of reference proteins usually fall in the range of 50 to 75%, whether evaluated by the slope–ratio technique (Hegsted et al., 1968) or by protein efficiency ratio (PER) (Neucere et al., 1972). Such tests are designed to assess the growth-promoting effects of proteins when they are present in the diet in growth-limiting concentrations. These do allow comparison of nutritional quality of one protein with that of another, but they give very little insight into the potential capacity of a protein to support an acceptable rate of growth.

The use of high dietary levels of proteins with unbalanced amino acid composition for growth of chicks, pigs, and rats was discussed by Carpenter and de Muelenaere (1965). They concluded that, under certain conditions, higher levels of poor proteins would result in nearly as good growth as could be obtained with practical diets containing good-quality proteins. However, in the studies reported, these authors used "groundnut flour (plus lysine)". They gave no details of the source or conditions of preparation of the flour nor of the lysine supplement.

Wethli et al. (1975) investigated the possibility of using groundnut meal without amino acid supplements as a source of additional protein in cereal-based diets of chicks. They reported that maximum growth rate could not be attained with groundnut meal even when very high dietary protein levels (43%) were used. Their conclusion was that the amino acids supplied by low-quality proteins were in such disproportion, compared with the animal's needs, that utilization of the first limiting amino acid(s) is impaired. Amino acid and aflatoxin content of the groundnut meal was reported.

Lysine and methionine (or total sulfur amino acids) are generally considered to be the most limiting amino acids in peanut protein for humans (FAO, 1965), chicks (Wethli et al., 1975), rats (Carpenter and Anantharaman, 1968), and rabbits (Spreadbury, 1974). McOsker (1962) evaluated the limiting amino acid sequence in raw and roasted peanuts using rats as the test animal and found threonine to be limiting also. In unroasted peanut paste, lysine, methionine, and threonine were equally limiting while in paste from roasted nuts the limiting sequence was lysine, threonine, and methionine. Calculations based on amino acid content of the peanut protein and rat amino acid requirements indicated that threonine should not be limiting and thus McOsker concluded that about 30% of the threonine in peanut protein is not biologically available to the rat. Young et al. (1973) determined amino acid content of 16 varieties of peanuts. By comparing their data with FAO (1965) recommended levels they concluded that, in addition to the three amino acids already mentioned, isoleucine and valine might also be limiting.

The source and history of peanuts and peanut meal used in estimates of biological value of peanut protein are not given in many of the studies reported (e.g., Hegsted et al., 1968; Carpenter and de Muelenaere, 1965; and Wethli et al., 1975). Such factors as variety, maturity, post-harvest drying conditions, temperatures attained during transportation and storage, and defatting processes can affect protein quality of peanut meal. In the experiments reported here post-harvest deterioration of peanut protein quality was minimized and peanuts of known variety and

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production conditions were used. The peanut meal was fed at several dietary levels as the sole source of protein to weanling rats. Initial results showed that this meal was capable of producing growth rates equivalent to those attained with methionine-supplemented casein. Subsequently, the most limiting amino acids in the meal were ascertained by supplementing a diet containing suboptimum levels of the meal with individual and combinations of amino acids.

MATERIALS AND METHODS

Peanuts of the Florunner variety were grown near Plains, Georgia, using standard cultural practices. A decline in free arginine content during maturation of peanuts was used to predict date of harvest for optimum yield and market grade as described by Young et al. (1973). The harvested peanuts were dried to about 10% moisture at 35 °C, shelled on a federal-state inspection service sheller, and graded over a $^1/_2$ in. by $^3/_4$ in. slotted screen. Skins were loosened by holding the nuts at 40 °C for 2 or 3 days and then removed with a Model EX Ashton Food Machinery blancher. About one-half of the oil was expressed from the blanched nuts by passing them three times through a Carver press, and the residue was further extracted with commercial grade hexane at room temperature. The remaining peanut meal was air dried and ground in a Wiley mill to pass a 1-mm screen. At all times between harvest and incorporation of the meal into animal diets, the peanuts or meal were held at 4 °C, 50% R.H. when not in use.

For amino acid analysis, samples of the peanut meal and casein were hydrolyzed by a modification of the method of Roach and Gehrke (1970). In screw capped tubes, 100 mg of peanut meal or 25 mg of casein in 20 mL of 6 N HCl were flushed with nitrogen and heated at 145 °C for 0.5, 1, 2, 4, and 8 h. The pH was then adjusted to 2.1–2.2 with 12 N NaOH and the sample diluted to 50 mL with citrate buffer at pH 2.2. Amino acids were quantitated by ion-exchange chromatography as described by Spackman et al. (1958) using a Durrum Model D-500 with a 1.75 mm × 48 cm column packed with Durrum high-resolution cation exchanger (bead diameter, 8 ± 1 μm). Running time, including regeneration period, was 70 min. Amino acid content was corrected to zero hydrolysis time by extrapolation.

Nitrogen content of the peanut meal and casein was determined by Kjeldahl analysis, and residual oil content of the peanut meal was assessed by ether extraction on a Goldfisch apparatus. Composition of the peanut meal and casein is shown in Table I. Protein content was calculated as 6.38 × N for casein and 5.46 × N for peanut meal.

Each of the protein sources was incorporated into animal diets to provide five levels of dietary protein. In the casein diets these ranged from 8 to 24% in increments of 4% and in the peanut diets the range was 6.7 to 20% at 3.3% intervals. The casein diets were supplemented with *l*-methionine at 1.4% of the casein. All diets contained 2.2% Vitamin Fortification Mixture (ICN Nutritional Biochemical Corp.), 3.5% salt mixture (Williams et al., 1968), and 1.5% cellulose. The casein diets had 8% corn oil. A sufficient amount of the expressed, filtered oil from peanuts was added back to those diets to give them a total of 8% oil content. Equal parts of sucrose and dextrin were added to make the diets to 100%. In the second experiment, crystalline *l*-amino acids were added to the diet containing 13.3% peanut protein as indicated in Table III.

Weanling male Sprague-Dawley rats (Charles River Breeding Laboratories) were fed stock diet and water overnight and then allotted to dietary groups. Initial mean weights of the groups of animals in each experiment varied by less than 1.5% and standard deviations of the means were less than 10%. The animals were housed individually in stainless steel cages with mesh floors and provided with food and deionized water ab libitum. Food consumption was measured three times per week and weight gain once each week. There were ten animals per group in the first experiment which continued for 4 weeks and eight rats for each dietary treatment in the second study which lasted for 2 weeks.

Table I. Nitrogen and Amino Acid Content (%) of Florunner Peanut Meal and Casein as Used in Rat Diets

	Peanut meal[a]	Casein
Nitrogen (Kjeldahl)	8.05	13.6
Aspartic acid	6.40	6.83
Threonine	1.39	3.89
Serine	2.85	5.50
Glutamic acid	8.29	17.17
Proline	2.67	13.30
Glycine	3.25	1.75
Alanine	2.06	2.87
Cystine	0.79	1.14
Valine	1.96	5.79
Methionine	0.49	2.91
Isoleucine	1.88	5.03
Leucine	3.42	8.85
Tyrosine	2.24	5.78
Phenylalanine	2.88	4.95
Histidine	1.56	5.70
Lysine	2.00	7.27
Arginine	6.76	4.24
Total amino acids[b]	50.90	103.0

[a] Residual oil content of the peanut meal was 8.93%. [b] Total weight of hydrolyzed amino acids is approximately 15% greater than that of the protein from which they are derived because of the addition of a molecule of water for each peptide bond hydrolyzed.

RESULTS AND DISCUSSION

The content of some amino acids in the peanut meal diets are compared with published requirements for albino rats (National Academy of Sciences, 1972) in Table II. In each diet, amino acids present at less than 90% of requirement are underlined. From these data, sulfur amino acids appear to be the most limiting in this peanut meal. About one-third to one-half of the dietary need for sulfur amino acids can be met by cystine (National Academy of Sciences, 1972). Thus the diet containing 20% peanut protein provided between 66 and 82% of the published requirement for sulfur amino acids for the growing rat. Lysine and threonine would seem to be the next two most limiting amino acids in that order, since each is supplied to about 75% of its requirement by the diets containing 16.7 and 13.3% protein, respectively.

Growth of the animals vs. protein content of the diets for the first experiment is shown in Figure 1. Rats fed the diet containing 20% of peanut protein had gained as much weight as animals fed 12 to 24% casein diets at the end of 2 weeks (Figure 1a). At this time there was a significant difference between weight gained by rats fed the 16.7% peanut protein diets and those consuming the 24% casein diet. By the end of 4 weeks (Figure 1b) this difference was no longer significant and 16.7 or 20% of dietary peanut protein resulted in growth equivalent to that of 12% or more of casein protein in the diet.

Rats fed the diets containing either 16.7 or 20% protein from the peanut meal gained weight at a rate of about 6 g/day during the first 2 weeks and about 7.5 g/day over the whole 4-week period of the feeding trial. Such rapid growth, comparable with that obtained with methio-

Table II. Amino Acid Content of Diets Containing Florunner Peanut Meal and Requirements of the Rat as % of Total Diet

		% of peanut protein in diet				
	Requirement[a]	20.0	16.7	13.3	10.0	6.7
Arginine	0.67	3.08	2.57	2.05	1.54	1.03
Histidine	0.33	0.71	0.59	0.47	0.36	0.24
Isoleucine	0.61	0.86	0.71	0.57	0.42	0.29
Leucine	0.83	1.56	1.30	1.04	0.78	0.52
Lysine	1.0	0.91	0.76	0.61	0.45	0.30
Methionine	0.67	0.22[b]	0.19	0.15	0.11	0.07
Cystine		0.36	0.30	0.24	0.18	0.12
Phenylalanine	0.89	1.31	1.09	0.88	0.66	0.44
Tyrosine		1.02	0.85	0.68	0.51	0.34
Threonine	0.56	0.64	0.53	0.43	0.32	0.21
Valine	0.67	0.89	0.75	0.60	0.45	0.30

[a] From National Academy of Sciences (1972). [b] Underlined values indicate that the diet contains less than 90% of the requirement for that amino acid.

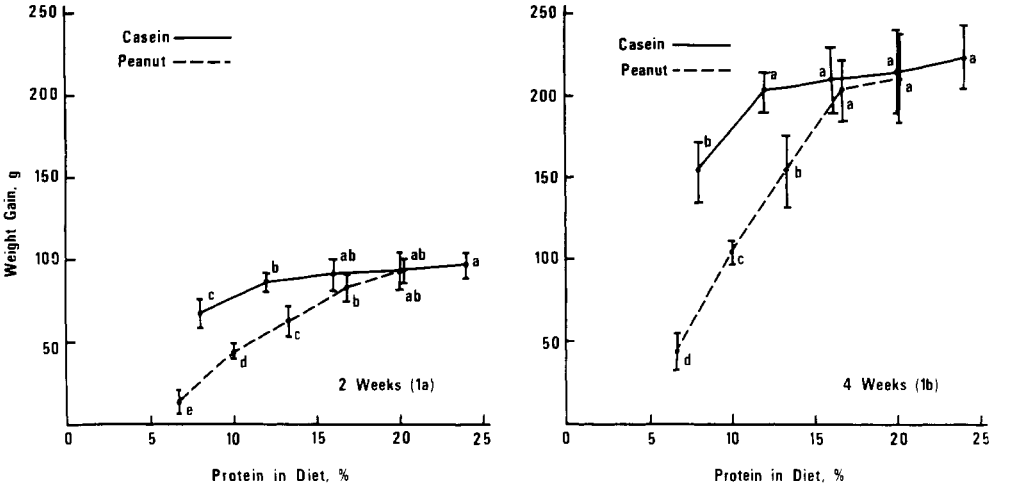

Figure 1. Weight gain of rats fed several dietary concentrations of either defatted peanut meal or casein as a protein source for (a) 2 weeks or (b) 4 weeks. The vertical lines above and below each point represent ±1 standard deviation of the mean. The lower case letters following each point indicate statistical difference of means according to Duncan's (1955) multiple range test. Means not followed by a common letter are different at $P \leq 0.05$.

nine-supplemented casein diets, tends to refute the evidence of amino acid deficiency in these diets that is indicated by the data of Table II. Dietary amino acid deficiency depresses food comsumption, and this was quite evident in rats fed the two lower levels of peanut protein (Figure 2). However, animals given diets with the two higher levels of peanut meal consumed more feed than those fed comparable levels of casein protein. The amino acid requirements of rats are tabulated in terms of dietary concentration on the assumption of normal food intake (National Academy of Sciences, 1972). Dietary treatments that result in increased food intake, such as the higher levels of peanut meal used in this study, may satisfy absolute requirements for amino acids even though their concentration in the diet is less than that recommended.

The sequence of limitation of amino acid supply predicted by the data of Table II also were not wholly substantiated by the results of the second study (Table III), in which diets containing 13.3% peanut protein were supplemented with methionine, lysine, and threonine. The amino acid analysis indicated that this diet contained about 50% of the methionine, 60% of the lysine, and about 75% of the threonine needed by the rat. Therefore, a response to methionine and an additional response to the combination of methionine and lysine would have been expected. However, the data (Table III) show that no one of the three amino acids alone nor any possible combination of two of the three amino acids added to the diet significantly improved performance of the rats over that obtained with the unsupplemented diet. Only when

Table III. Animal Weight Gain and PER of Diets Containing 13.3% Peanut Protein Supplemented with Methionine, Lysine, and Threonine and Fed to Weanling Rats for 2 Weeks[a]

	Weight gain, g	PER
Basal	60.0[2]	2.76[2]
Basal + Met[b]	65.8[2]	2.81[2]
Basal + Lys	59.7[2]	2.67[2]
Basal + Thr	59.7[2]	2.82[2]
Basal + Met + Lys	53.6[2]	2.62[2]
Basal + Met + Thr	63.0[2]	2.67[2]
Basal + Lys + Thr	58.0[2]	2.74[2]
Basal + Met + Lys + Thr	88.4[1]	3.46[1]

[a] Values in a column having no common superscript are significantly different at $P \leq 0.01$ according to Duncan's (1955) multiple range test. [b] Amino acids were added as *l* isomers in the following percentages of the total diet: methionine, 0.21; lysine, 0.24 (as lysine HCl, 0.30); and threonine, 0.14.

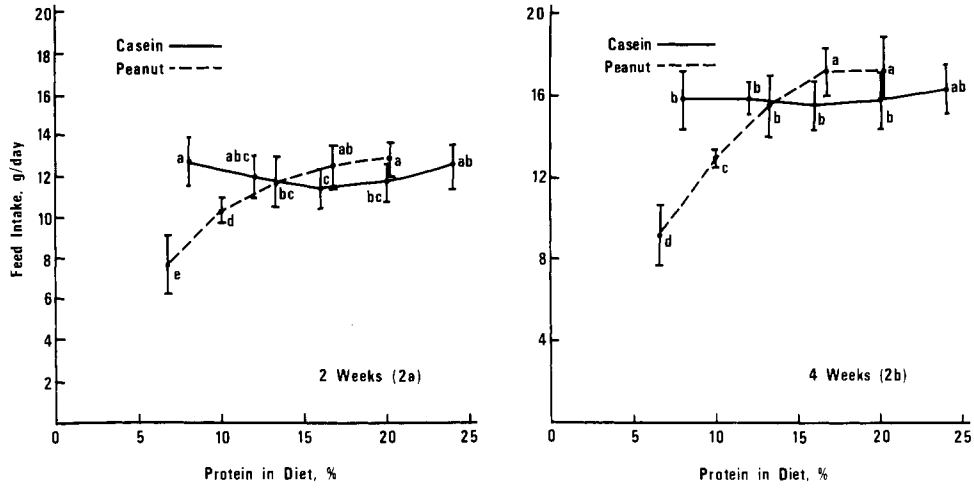

Figure 2. Average daily feed intake of rats fed several dietary concentrations of either defatted peanut meal or casein as a protein source for (a) 2 weeks or (b) 4 weeks. The vertical lines above and below each point represent ±1 standard deviation of the mean. The lower case letters following each point indicate statistical difference of means according to Duncan's (1955) multiple range test. Means not followed by a common letter are different at $P \leq 0.05$.

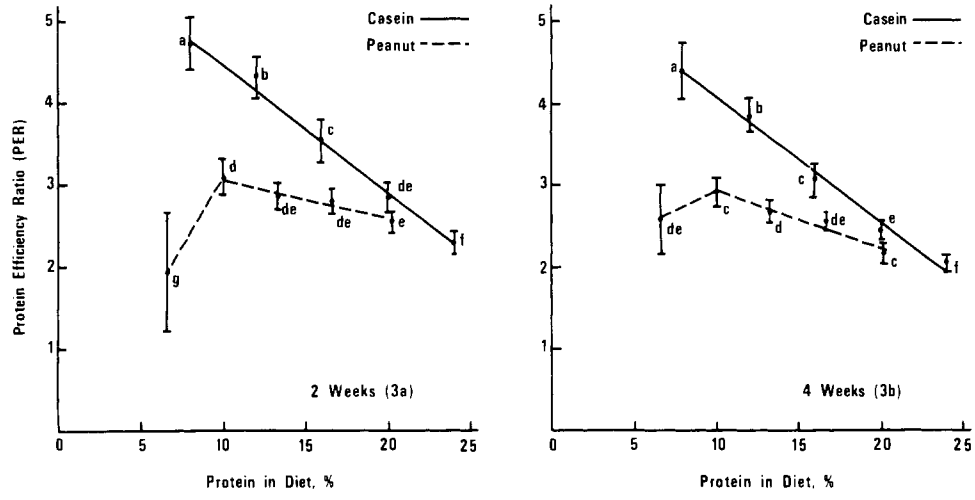

Figure 3. Protein efficiency ratio of defatted peanut meal and casein fed to rats at several dietary concentrations for (a) 2 weeks and (b) 4 weeks. The vertical lines above and below each point represent ±1 standard deviation of the mean. The lower case letters following each point indicate statistical difference of means according to Duncan's (1955) multiple range test. Means not followed by a common letter are different at $P \leq 0.05$.

methionine, lysine, and threonine were all added to the 13.3% peanut protein diets were increases in weight gain and PER statistically significant. With supplementation of all three amino acids, weight gain of the animals was comparable with that indicated by the curve in Figure 1a for rats fed diets containing a similar concentration of casein.

McOsker (1962) reported quite similar results. Amino acid analysis indicated that methionine should be more limiting than lysine and that threonine should not be limiting in a diet containing 15% protein supplied by blanched peanuts. Rat bioassay showed, however, that the three amino acids were equally limiting. In partial explanation of these results, McOsker presented evidence which he interpreted as indicating that about 30% of the threonine of peanut protein was biologically unavailable to the rat.

The free ϵ-amine group of lysine is known to be highly reactive (Carpenter, 1973). Many of the addition products thus formed are not hydrolyzed by the digestive systems of monogastric animals and therefore render the lysine biologically unavailable. Since the rate of reactions involving the side chain of lysine is very sensitive to temperature, the peanuts and peanut meal used in this study were carefully handled to avoid any unnecessary exposure of the product to elevated temperatures. However, the short heating period required for blanching may have been sufficient to bring about some reaction. It is also possible that some of the lysine of peanut proteins is naturally present in a form that is unavailable.

The protein inadequacy of the diet containing 13.3% peanut protein, compared with those containing 16.7 and 20% protein, thus can be attributed not only to the lower concentration of amino acids in the diet but also to the decreased feed consumption. These two factors combined to reduce intake of one or more amino acids below a critical level necessary for maximum growth. The bioassays (Table III) indicate that methionine, lysine, and threonine were equally limiting and, therefore, that some of the lysine and threonine are biologically unavailable.

Values for PER of the diets varied with protein level and time (Figure 3). For any one protein, PER will increase from very low dietary protein concentrations to a peak value at a protein level that is yet too low to promote maximum growth rate. From this peak then, PER declines linearly as dietary protein concentration is increased

(Buamah and Singsen, 1976). In this study the maximum PER for peanut meal occurred with the diets containing 10% protein. The relative value for peanut protein at this concentration, compared with the value for 10% casein indicated by the curves in Figure 3, is approximately 68% at 2 weeks and 71% at 4 weeks. These values for relative PER of peanut protein would be 13% lower if the factor of 6.25 had been used to calculate protein concentration from nitrogen content as is often done (AOAC, 1975). However, a conversion factor of 5.46 is considered to be more nearly correct for the proteins in peanuts (USDA, 1957).

The data presented show that peanut meal as the sole source of protein is capable of producing maximum rate of growth in weanling rats. The meal used was carefully handled to minimize post-harvest changes in peanut protein quality. The failure (Wethli et al., 1975) to obtain maximum growth of chicks with groundnut meal may have been due to their higher dietary protein requirement or to deterioration of protein quality of the product during processing and storage.

Peanut meal, prepared and stored under conditions to minimize deterioration of protein quality, could become a significant source of protein on the world food market. In some geographical areas where the supply of protein is scarce, the peanut residue from oil mills is essentially wasted because of unsanitary conditions that prevail during storage and processing. Recognition of the value of the protein of the press cake might provide the economic incentive to improve processing conditions. The meal has potential as a human food but it might well serve as a source of feed for production of meat animals in these areas. Even if growth rate of such animals was not maximal, this would be nutritionally preferable to a lack of animal proteins in the diets of the people.

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Lead and Other Elements in Sheep Fed Colored Magazines and Newsprint

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Colored paper from magazines and newsprint was fed as 23% of a ration to sheep for 124 days. Among 44 elements determined in the paper and selected sheep tissues, marked accumulation of lead in animal organs was found reaching 22 ppm in bone. Barium and bromine was higher in kidney and fluorine was higher in bone of sheep fed the paper vs. the control ration. Histological examination of animal tissue revealed no apparent lesions which could be attributed to consumption of the paper diet. The sheep on the paper ration consumed more and gained weight at a faster rate than the control animals. The paper rations showed a higher in vivo digestibility than the control diet.

Several investigators have incorporated waste paper in farm animal rations as a possible substitute form of cellulose in their diets. Others have studied its digestibility in vitro. Waste paper has included newsprint and magazines (Sherrod and Hansen, 1973; Rural Research, 1974; Dinius and Oltjen, 1971; Daniels et al., 1970; Dinius and Oltjen, 1972; Mertens et al., 1971; Kesler et al., 1967), office bond (Nishimuta et al., 1969), and a variety of others (Becker et al., 1975). Polychlorinated biphenyls (PCB's) (Shahied et al., 1973; Masuda and Kagawa, 1972; Villeneuve et al., 1973), polychlorinated terphenyls (Thomas and Reynolds, 1973; Villeneuve et al., 1973), and unidentified compounds (Serum, et al., 1973) have been detected in waste paper and paperboard. Lactating cows fed waste newsprint, grey or brown cardboards, or computer paper as 30% of their ration for periods up to 39 days excreted PCB's in their milk and showed tissue

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