Protein Nutritional Quality of Meal Made From Several Cultivars of Peanuts as Measured by Rat Bioassay¹ Josephine Miller, R. Dixon Phillips and C.T. Young²

ABSTRACT

Peanut protein supported rapid growth in weanling rats when present in the diet in sufficient quantity. Defatted meal was prepared by cold pressing and hexane extraction of oil from seeds of Florunner, Tifrun, UF 70115, Tennessee Red, Tamnut and Comet cultivars of peanuts. A concentration of 16% peanut protein resulted in better growth than 12% protein from all cultivars tested. Increasing protein to 20% of the diet further improved growth of rats fed meal from some, but not all, cultivars of peanuts. Apparent digestibility of nitrogen in the meals was about 92%. Peanut meal is potentially a good source of protein for animal production if the peanuts and meal are properly handled after harvest to maintain the nutritional quality of the product. Some statistically significant differences occurred in growth performance of rats fed meals from the several cultivars of peanuts but these are not likely to be of practical importance. However, they suggest that protein quality of the peanut might be improved by breeding.

The nutritional quality of peanut protein is considered to be low because the concentration of several of the essential amino acids is below optimal levels for promoting growth of young animals. When compared with casein in the accepted bioassay for protein efficiency ratio (PER, Neucere et al., 1972) or by the slope-ratio technique (Hegsted et al., 1968), the biological value is commonly found to be 50 to 75% of that of the standard protein. Such tests are conducted with growth-limiting levels of dietary protein and provide little information on the potential capacity of a protein to support an acceptable rate of growth. Carpenter and de Muelenaere (1965) concluded that, under certain conditions, higher levels of poor-quality proteins would result in nearly as good growth of chicks, pigs, and rats as could be obtained with practical diets containing good-quality proteins. Defatted meal from Florunner peanuts, when incorporated into diets to provide 16.7% protein, supported growth of weanling rats at a rate comparable to that obtained with diets containing 12% to 24% of casein (Miller and Young, 1977).

This paper describes results of a study similar to that mentioned above using meal from several cultivars of peanuts that are of current commercial or genetic interest.

Keywords: Protein nutritional quality, rat bioassay, peanut meal, amino acids, groundnut.

Materials and Methods

All peanuts were of the 1976 crop except for one lot of Florunner which was grown in 1975 and had been kept at 5^{0} C since curing. The Tifrun and Florunner '76 peanuts were grown in adjacent plots in Tift County, Georgia. Florunner '75, Tennessee Red and Tamnut were produced in Sumter County and UF 70115 in Lee County, Georgia. Comet peanuts were grown in Caddo County, Oklahoma.

¹This work was supported in part by the Georgia Agricultural Commodity Commission for Peanuts.

²Associate Professor and Assistant Professor, Food Science Department, University of Georgia College of Agriculture Experiment Stations, Georgia Station, Experiment, Georgia, 30212 and Associate Professor, Food Science Department, North Carolina State University, Raleigh, North Carolina, 27607 respectively.

Peanuts that had been subjected to a minimum of post-harvest rain were dried with controlled heat and brought into the laboratory as soon as possible. A 30-pound capacity oven (Preedit Model 37, Electric Roaster Company, Erie, PA) was preheated to 157°C and loaded with 25 to 27 pounds of shelled peanuts at room temperature. Peanuts were heated in the rotating oven until air temperature of the oven returned to 105°C. The nuts were cooled rapidly in a draft of ambient air and skins were removed with a model EX Ashton Food Machinery blancher. About one-half of the oil was pressed out of the blanched nuts by passing them three times through a Carver press. The final hexane extraction was carried out by passing solvent through the ground, pressed meal in chromatography columns (8 cm diameter) connected to vacuum. One-kilogram batches of the meal, which filled the columns to about 50 cm, were washed with approximately 2.5 liters of hexane. The columns remained connected to the vacuum overnight and the last of the visible solvent was eluted from the meal during the night. The meal was spread on a tray in a hood and stirred several times during the next 24 hours to allow the remaining hexane to evaporate. Peanuts, peanut meals and mixed diets were kept at 5^{0} C at all times when not in use.

Each of the peanut meals was incorporated into three diets which contained 12, 16 and 20% protein, respectively, calculated as 5.46 times the nitrogen content of the meal. All diets contained 2.2% vitamin mix (Vitamin Fortification mixture, ICN Nutritional Biochemical Corp.), 3.5% salt mix (Cohen et al., 1967) and sufficient peanut oil to make the total fat content of the diet, including that remaining in the meals, to 8%. Corn starch was added to 100%. Casein (ANRC, Humko Sheffield, Lyndhurst, New Jersey) was used as the standard protein (N x 6.38).

Weanling male rats (Sprague-Dawley CD^R, Charles River Breeding Laboratories, Wilmington, Massachusetts) were housed individually in cages with wire mesh floors. Feed and deionized water were provided ad libitum to 10 rats per dietary treatment. Feed intake was measured every second day and animals were weighed every seven days. PER was calculated as grams of weight gained per gram of protein consumed.

Feces from each individual rat were collected from the 17th through the 21st days of the study in partitioned trays with fine wire mesh bottoms. They were stored in a freezer until collections were complete. Feces from rats consuming the same diets were pooled and dried for 24 hours at 100^{0} at which point no further loss of water was noted. Food and hair were separated from the fecal particles by gentle shaking on a screen in a stream of air and the dry weight of the collection was determined. The pooled samples were ground in a Wiley Mill and the fecal powder sampled for nitrogen and moisture determinations.

Nitrogen content of the peanut meals, diet mixtures and fecal powders were determined by Kjeldahl analysis. Residual oil content of the meals was measured by weighing material extracted by the Bligh and Dyer (1959) method. Apparent nitrogen digestibility (nitrogen consumed minus nitrogen in the feces divided by nitrogen consumed) was calculated for each diet based on the four-day fecal output and corresponding diet intake.

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 6N HCI were flushed with nitrogen and heated at 145⁰ for 0.5, 1, 2, 4 and 8 hr. The pH was then adjusted to 2.1 - 2.2 with 12N NaOH and the sample diluted to 100 ml with citrate buffer at pH 2.2. Amino acids were quantified by ionexchange chromatography as described by Spackman et al. (1958) using a Durrum Model D-500 with a 1.75 mm x 48 cm column packed with Durrum high-resolution cation exchange resin (bead diameter, 8 + 1 microns). Running time including regeneratin period, was 70 minutes. Amino acid content was corrected to zero hydrolysis by extrapolation.

Results and Discussion

Weight gain for rats fed casein and the peanut meals at the three dietary protein levels are shown in Table 1. A significant difference ($P \le 0.01$) in weight gain between rats fed diets with 12% protein and those fed the 16% dietary protein levels was

Table 1. Weight gain (g) of rats fed diets containing peanut protein at three different concentrations*

Cultivar	Dietary pr 12%	centration 20%	
Florunner '75	92 by	134 bx	137 bx
Florunner '76	94 by	137 bx	138 bx
Tifrun	90 Ьу	133 bx	134 bx
UF 70115	91 by	123 bx	140 bx
Tennessee Red	93 bz	128 by	150 abx
Tamnut	81 bz	122 by	145 abx
Comet	82 bz	120 by	148 abx
Casein	146 ay	164 ax	160 ax

*a,b;means in a column not having a common letter are significantly different at $p \le 0.01$ and x,y,z; means in a row not having a common letter are significantly different at $p \le 0.01$ according to Duncan's (1955) multiple range test.

 Table 2. Protein efficiency ratio of peanut protein fed at three different concentrations*

	Dietary protein concentration					
Cultivar	12%	16%	20%			
Florunner '75	2.54 b	2.43 b	2.10 b			
Florunner '76	2.52 b	2.34 bc	2.02 b			
Tifrun	2.38 bc	2.30 bc	2.02 b			
UF 70115	2.41 bc	2.32 bc	2.02 b			
Tennessee Red	2.41 bc	2.22 c	2.11 b			
Tamnut	2.33 c	2.26 bc	2.08 b			
Comet	2.30 c	2.18 c	2.08 b			
Casein	3.64 a	2.86 a	2.35 a			

*Values in a column having no common letter are significantly different at $P \leq .01$ according to Duncan's (1955) multiple range test.

observed for all protein sources. However, the differences between weight gains associated with these two protein concentrations was much greater for rats fed the peanut meals than for those given casein diets. This indicates that the adequacy of the amino acid supply was considerably improved by increasing the dietary concentration of the peanut protein from 12 to 16%. For casein and four of the peanut sources no additional increase in weight gain was obtained bv further increasing the dietary protein concentration to 20%. This final increment of dietary protein did bring about further weight gain in rats fed meal derived from Tennessee Red, Tamnut and Comet peanuts, however. At the highest level of dietary protein, there was no difference between weight gain by rats fed these three peanut sources and those fed the 20% casein diet.

At the lowest level of dietary protein used in this study, the PER values (Table 2) for the peanut meals ranged from 64 to 70% of the case in diet. At a dietary protein concentration of 20%, the biological value of the peanut meals was approximately 85% that of the standard protein, case in. Some differences between the PER values for peanut meals fed at either 12 or 16% of the diet were statistically significant, but these may be of little practical value.

Apparent digestibility of the nitrogen of the diets used in this study is shown in Table 3. The value of aproximately 95% obtained for the casein diet is similar to published data (Lahiry et al., 1977). For all peanut sources except the 1975 Florunner crop, apparent digestibility of the dietary nitrogen was about 92%. Carpenter and Anantharaman (1968) reported an apparent digestibility of about 83% for

Table 3. Apparent nitrogen digestibility of peanut protein fed at three different concentrations*

Cultivar	<u>Dietary</u> 12%	<u>concent</u> 16%	<u>ration</u> 20%	Cultivar mean		
Florunner '75	.910	.906	.910	.908 c		
Florunner '76	.919	.921	.918	.919 b		
Tifrun	.919	.928	.930	.925 b		
UF 70115	.918	.925	.9 28	.926 b		
Tennessee Red	.919	.927	.922	.923 b		
Tamnut	.923	.926	.927	.925 b		
Comet	.924	.923	.927	.924 b		
Casein	.941	.952	.956	.949 a		

*Values not followed by a common letter are significantly different at P ≤ .01 according to Duncan's (1955) multiple range test. nitrogen from peanut meal. These authors gave little information on the source and preparation of the meal, and differences in handling of the material prior to feeding may account for much of the observed difference in apparent digestibility between the two studies. The value of 91% obtained for apparent digestibility of nitrogen in the Florunner '75 sample was statistically different from that of the other peanut sources. This slightly lower digestibility could be the result of some deterioration in the sample with time even though the peanuts were held at 5°C for most of the time after harvest.

Table 4. Amino acid content of diets containing 12% protein from several cultivars of peanuts and requirements of the rat as % of the total diet.

	Requirementa	FR'75 ^b	FR'76	Tifr	UF	TR	Tamn	Comt	Casein
Arginine	0.67	1.85	1.91	1.86	1.90	1.82	1.88	1.81	0.56
Histidine	0.33	0.46	0.47	0.48	0.51	0.45	0.48	0.47	0.51
Isoleucine	0,61	0.48 ^c	0.50	0.49	0.51	0.47	0,51	0.50	0.75
Leucine	0.83	0.94	0.97	0.95	0.99	0.92	0.95	0.92	1.34
Lysine	1.0	0.52	0.52	0.48	0.51	0.46	0,49	0.47	1.14
Methionine	0.67	0.14	<u>0.14</u>	0.14	0.15	0.15	0.14	0.13	0.62 ^d
Cystine		0.16	0.17	0.16	0.17	0.17	0.15	0.15	0.05
Phenylalanine	0.89	0.74	0.76	0.76	0,80	0.73	0.79	0.77	0.72
Tyrosine		0.53	0.55	0.54	0.56	0.56	0.58	0.55	0.79
Threonine	0.56	0.39	0.39	0.40	0.42	0.38	0.40	0.40	0.62
Valine	0.67	0.57	0.57	0.60	0.62	0.60	0.62	0,62	0.89

^aNational Academy of Sciences, "Nutrient Requirements of Laboratory Animals," 2nd ed., Washington, DC 1972, p. 64.

^bAbbreviations are: FR'75, Florunner '75; FR'76, Florunner '76; Tifr, Tifrun; UF, UF70115; TR, Tennessee Red; Tamn, Tamnut; Comt, Comet.

 $^{\rm C}{\rm Underlined}$ values indicate that the diet contains less than 90% of the requirement for that amino acid.

 $^{\rm d} Including \ {\rm supplemental}$ l-methionine added to the diet.

Amino acid composition of meals made from the several cultivars of peanuts is indicated in Table 4 in which data are presented as the amount of essential amino acids provided by 12 grams of peanut protein. Differences among the cultivars in content of these amino acids per unit of protein were small but the meals did differ somewhat in total nitrogen content. Values obtained by Kjeldahl analysis were: Florunner '75, 8.7% N; Florunner '76, 9.3%; Tifrun, 9.5%; UF 70115, 9.2%; Tennessee Red, 10.6%; and Comet, 9.9%. Amino acid requirements of the growing rat are shown in Table 4 for comparison. Lysine and methionine were the most limiting amino acids and were not supplied at recommended levels even in diets containing 20% peanut protein. Despite these deficiencies the animals grew quite well on the highest level of dietary protein and, if the experiment had been continued longer, would likely have matched the weight achieved by those animals fed the case in control diet (Miller and Young, 1977). Threonine, isoleucine, and valine (in that order) were the next most limiting amino acids but were supplied in adequate quantity by diets containing 16% peanut protein.

Data presented in this paper indicate as suggested before (Miller and Young, 1977) that peanut meal, if the peanuts and the meals are properly handled, can be a valuable source of dietary protein for humans or for production of meat animals to be consumed by humans. Differences in performance of rats in this study fed meals from several peanut cultivars may be of no practical significance at this time. However, they do suggest that a potential does exist for improving nutritional quality of their proteins by breeding. Performance of the rats was not adequately predicted by comparison of values for amino acid content of the peanut protein with published requirements (National Academy of Sciences, 1972) for this animal. This should serve as a caution against making a judgment about the quality of a protein without testing it in a biological system.

Acknowledgments

Peanuts for this study were donated by R.O. Hammons of the Coastal Plains Experiment Station, Tifton, GA; Carter's Warehouse, Plains, GA; the Foundation Seed Center, Plains, GA; and Gold Kist Co., Anadarko, OK. The food grade corn starch used was a gift from Corn Products Co.

The technical assistance of Richard Stinchcomb, Joy Adams, and Rosa Mathews is gratefully acknowledged.

Literature Cited

- 1. Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.
- 2. Carpenter, K.J., and K. Anantharaman. 1968. The nutritional value of poor proteins fed at high levels. 1. Growth of rats. Br. J. Nutr. 22:183-197.
- Carpenter, K.J., and H.J. de Muelenaere. 1965. A comparative study of performance of high-protein diets of unbalanced amino acid composition. Proc. Nutr. Soc. 24:202-209.
- 4. Cohen, N.L., Reyes, J.T. Typpo, and G.M. Briggs. 1967. Vitamin B_{12} deficiency in the golden hamster. J. Nutr. 91:482-488.
- 5. Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11:1-42.
- Hegsted, D.M., R. Neff, and J. Worcester. 1968. Determination of the relative nutritive value of proteins. Factors affecting precision and validity. J. Agric. Food Chem. 16:190-193.
- Lahiry, N.L., L.D. Satterlee, H.W. Hsu, and G.W. Wallace. 1977. Characterization of the chlorogenic acid binding fraction in leaf protein concentration. J. Food Sci. 42:83-85.
- Miller, J., and C.T. Young. 1977. Protein nutritional quality of Florunner peanut meal as measured by rat bioassay. J. Agric. Food Chem. 25:653-657.

- 9. National Academy of Sciences, "Nutrient Requirements of Laboratory Animals," 2nd ed., Washington, D.C. 1972, p. 64.
- Neucere, N.J., E.J. Conkerton and A.N. Booth. 1972. Effect of heat on peanut proteins. II. Variations in nutritional quality of the meals. J. Agric. Food Chem. 20:256-259.
- 11. Roach, D., and C.W. Gehrke. 1970. The hydrolysis of proteins. J. Chromatog. 52:393-404.
- 12. Spackman, D.H., W.H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in chromatography of amino acids. Anal. Chem. 30:1190-1206.