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### BIOLOGICAL EVALUATION OF PARA-RUBBER SEEDS (*Hevea braziliensis*)

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#### ABSTRACT

Proximate nutrient composition and mineral constituents were determined in full-fat and defatted rubber seeds. Total amino acid composition of full-fat rubber seeds, peanut and soybean meals was also determined by column chromatography for amino acids. Four experiments were conducted using 28-day old albino rats (initial weights, 50-55 g) with the following objectives: (i) to compare the protein quality of full-fat and defatted rubber seed meals; (ii) to test the effect of autoclaving on the protein quality of full-fat and defatted rubber seed meals; (iii) to assess the effects of hot water, dilute acid and dilute alkali extraction on the nutritive value of rubber seed and (iv) to measure responses to amino acid supplementation of both full-fat and defatted rubber seed meals.

The full-fat and defatted rubber seeds had protein contents of  $22.54 \pm 0.52\%$  and  $36.48 \pm 0.84\%$  respectively and fat contents of  $49.49 \pm 1.54\%$  and  $8.54 \pm 0.38\%$ . Both the full-fat and defatted samples contain fairly high levels of calcium ( $0.48 \pm 0.02$  and  $0.88 \pm 0.05\%$ ); phosphorus ( $0.64 \pm 0.06$  and  $0.94 \pm 0.09\%$ ); potassium ( $0.96 \pm 0.42$  and  $1.54 \pm 0.63$ ); iron ( $12.72 \pm 11.54$  and  $147.45 \pm 8.32$  mg/kg) and zinc ( $78.46 \pm 2.11$  and  $112.29 \pm 4.86$  mg/kg). Amino acid analysis indicated lower levels of lysine,  $\beta$ -leucine, leucine, phenylalanine, tyrosine, proline and glycine compared to peanut and soybean and lower methionine and cystine content compared to soybean. Poorer protein quality indices were obtained for both types of rubber seed compared to peanut and soybean, while the full-fat seed meal was nutritionally superior to the defatted sample. Autoclaving did not improve the nutritive indices of the rubber seed meals. Dilute acid or alkali treatment worsened rat responses, while hot water extraction resulted in nonsignificant improvements in the nutritive value. Responses to amino acid supplementation suggest that lysine and methionine are most limiting in rubber seed protein. The overall results also suggest an impairment of the nutritive value of rubber seeds by extraction with petroleum spirit.

#### INTRODUCTION

The rapidly increasing population continues to task the available protein resources. The situation is particularly acute in many areas of the tropics and subtropics where available supplies have

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repeatedly been shown to be disproportionately lower relative to the population. Yet in these areas, there is also an urgent need to expand livestock numbers as well as upgrade their performance in efforts to improve the animal protein availability situation. Feedstuffs play a key role in livestock productivity and the simple-stomached animals which have been recognised to have the greatest role to play in alleviating the animal protein shortage within the short run compete directly with man for the available food resources. Many protein sources, however, abound in these areas that have not found use as human foods, which could have potentials as feed sources for livestock. Effective utilisation has however been hampered by a lack of precise knowledge of their potential contribution of nutrients and processing conditions that could lead to optimization of these nutrients.

Para-rubber trees have been cultivated mainly for their latex and are vital to the economic survival of Ceylon and Malaysia. Nigeria is among the principal producers outside Asia. Present acreage are large and conscious efforts at increasing the size of commercial plantations are apparent. Improved technology has also resulted in the use of vegetative propagation methods rather than planting from seeds, which thus makes most of the seeds currently produced go to waste. It is estimated that a rubber tree produces about 2,000-2,500 seeds per season and each rubber tree remains productive for over 30 years. This gives an indication of the quantity of seeds that go to waste annually.

Studies on the nutrient content and utilization of rubber seed are limited in number and in scope (1,2,3,4). The present paper therefore reports on the biological evaluation of whole and defatted rubber seed and a survey of processing methods that could improve their satisfaction.

### MATERIALS AND METHODS

All the rubber seed samples used in these experiments were obtained from the University of Ibadan Teaching and Research Farm rubber plantation. These were collected during the fruiting season, shelled and stored in polythene bags which were kept refrigerated at  $-5^{\circ}\text{C}$  and withdrawn for use as required. The peanut meal sample used in the 1st experiment was obtained as commercial samples from the Kano Oil Mills, Kano, Nigeria. The soybean was obtained from the Benue-Plateau State Marketing Board, Jos. Further treatment of this source involving first coarse milling and then solvent extraction with petroleum ether (BP  $40-60^{\circ}$ ). Solvent extracted samples were then ground to pass through a BS 30 mm sieve and autoclaved at  $121^{\circ}\text{C}$  at 15 lb/sq in pressure for 20 minutes. Nutritional casein (British Drug Houses Ltd., England) was used as standard protein for comparison in the 1st experiment.

Rubber seed samples were analysed for their proximate constituents as well as calcium and phosphorus concentrations by AOAC procedures (5). Rubber seed, soybean and peanut meals were analysed for their total amino acid content after acid hydrolysis by column chromatography for amino acids using the Hitachi Perkin Elmer amino acid analyser. Details of the hydrolytic techniques and instrument

operation parameters have been described elsewhere (6,7). Tryptophan was chemically determined (8).

Male Weanling albino rats of the Wistar Strain obtained from specific pathogen-free stock of the Department of Veterinary Pathology, University of Ibadan were used in the biological evaluations. These were weaned at 21 days and placed on a commercial stock diet for rats (Pfizer Livestock Feeds (Nig)) until they were 28 days old, when rats weighing between 50 and 55 g were selected for use. In all experiments rats were placed at random on each treatment. Rats were housed in stainless steel metabolic cages (Associated Crates, Ltd., Stockport; England), where experimental feeds and water were supplied *ad libitum*.

In all experiments, a basal diet of the following percentage composition was used: corn starch, 60.0; sucrose, 10.0; glucose, 5.0; non-nutritive cellulose, 10.0; peanut oil, 10.0; vitamin mixture (9), 1.0; and mineral mixture (9), 4.0. In all instances, protein sources were incorporated into the basal diet at the expense of corn starch such that they provided approximately 10% crude protein on dry matter basis.

The protein quality parameters used in the evaluation of the different diets were protein efficiency ratio (PER); net protein utilization (NPU); biological value (BV) and true protein digestibility (TPD). The procedures used were essentially those described by the National Academy of Sciences-National Research Council (10). The study involved 4 consecutive animal experiments.

#### Expt. I.

The first experiment involved a comparison of the protein quality of full-fat and petroleum ether extracted para-rubber seed meals with those of commercial prepressed solvent extracted peanut meal, solvent extracted, heat treated soybean meal and nutritional casein.

#### Expt. II.

This experiment compared the protein quality of full fat and ether extracted rubber seeds autoclaved at 121° C at 15 lb/sq in pressure for either 0, 10, 20 or 30 minutes. Autoclaving was carried out in shallow metal trays covered with aluminium foil.

#### Expt. III.

The third experiment compared the effects of hot water, 0.01 molar hydrochloric acid and 0.01 molar sodium hydroxide extraction of both full fat and defatted rubber seeds on protein quality. The extraction procedure involved soaking 100 g batches of the rubber seed meals in 500 ml of the solvent and leaving for 5 hrs in a water bath set at 65° C. The beakers were stirred at regular intervals. The solvent fractions were removed by centrifuging. The dilute acid and alkali treated samples were washed several times with hot water, after which all samples were dried at 50° C for 12 hr. These were then re-ground and stored in screw-capped Kellner jars until used for the feeding trials. Protein sources were incorporated to provide 10% protein in the final diets.

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## Expt. IV.

In this experiment, the effects of addition of synthetic L-lysine (lys), DL-methionine (meth), L-isoleucine (ileuc) and L-tryptophan (try), singly or in combination to 10% protein diets containing either full-fat or defatted para-rubber seeds were studied. The supplemental levels were determined by a comparison of the amino grams of rubber seeds with the dietary requirements for growing rats (11). The amino acids were incorporated into the test diets at the expense of maize starch. The parameters and techniques used in the evaluation were as described in the preceding sections.

Biological data from these experiments were subjected to analysis of variance and where statistical significance was indicated treatment means were compared by the Duncan's new multiple range test (12).

## RESULTS

The proximate and mineral composition of full-fat and defatted whole rubber seeds are presented in Table 1. The defatted samples had generally higher proximate nutrient levels compared to the full-fat samples except for the removed fat. Rubber seeds both in the whole and defatted forms appear to contain fairly high levels of calcium, phosphorus and potassium as well as high concentrations of the minor elements iron and zinc. Defatting also resulted in a higher concentration of the nutritionally important elements per unit weight of material.

TABLE 1. Proximate and Mineral Composition\* of Full-Fat and Defatted Rubber Seeds

|                                   | Full-fat rubber seeds | Defatted rubber seeds |
|-----------------------------------|-----------------------|-----------------------|
| <u>Proximate Nutrients (% DM)</u> |                       |                       |
| Dry matter (%)                    | 86.46±1.46            | 91.45±1.87            |
| Crude protein                     | 22.54±0.52            | 36.48±0.84            |
| Crude fibre                       | 3.80±0.12             | 4.40±0.15             |
| Ether extract                     | 49.49±1.54            | 8.54±0.38             |
| Ash                               | 3.47±0.08             | 5.33±0.11             |
| Nitrogen-free extracts            | 20.70±0.48            | 45.25±0.74            |
| <u>Mineral Constituents</u>       |                       |                       |
| Calcium (%)                       | 0.48±0.02             | 0.88±0.05             |
| Phosphorus (%)                    | 0.64±0.06             | 0.94±0.09             |
| Potassium (%)                     | 0.96±0.42             | 1.54±0.63             |
| Sodium (%)                        | 0.09±0.01             | 0.21±0.03             |
| Magnesium (%)                     | 0.28±0.02             | 0.34±0.03             |
| Chloride (%)                      | 0.07±0.002            | 0.18±0.008            |
| Iron (mg/kg)                      | 92.72±11.54           | 147.45±8.32           |
| Manganese (mg/kg)                 | 23.24±0.86            | 24.78±1.14            |
| Zinc (mg/kg)                      | 78.46±2.11            | 112.29±4.86           |
| Copper (mg/kg)                    | 25.54±1.08            | 31.78±1.65            |

\* Values represent means and their standard deviations from duplicate analysis of four different batches of samples.

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The total amino acid composition of full-fat para-rubber seed is compared to those of soybean and peanut meals in Table II. Rubber seeds contain lower levels of lysine, iso-leucine, leucine, phenylalanine, tyrosine, proline and glycine compared to both these sources and lower methionine and cystine levels compared to soybean but not peanut meal. In fact the total sulphur amino acid levels in rubber seeds is slightly higher than found in peanut, while the tryptophan level is higher than in both peanut and soybean.

TABLE II. Comparative Total Amino Acid Composition\* of Para-rubber Seed, Peanut and Soybean Meals (g/16g N)

| Amino Acids              | Rubber Seed | Soybean Meal | Peanut Meal |
|--------------------------|-------------|--------------|-------------|
| Arginine                 | 10.24±0.042 | 7.57±0.032   | 12.30±0.114 |
| Histidine                | 2.03±0.014  | 2.68±0.026   | 3.04±0.042  |
| Isoleucine               | 3.38±0.006  | 4.58±0.024   | 3.58±0.028  |
| Leucine                  | 6.24±0.014  | 7.94±0.028   | 7.09±0.034  |
| Lysine                   | 3.34±0.024  | 6.32±0.014   | 3.90±0.054  |
| Methionine               | 1.08±0.052  | 1.25±0.042   | 0.91±0.074  |
| Cystine                  | 1.38±0.034  | 1.64±0.048   | 1.28±0.038  |
| Methionine + cystine     | 2.46±0.048  | 2.89±0.061   | 2.19±0.084  |
| Phenylalanine            | 4.94±0.074  | 5.68±0.102   | 5.60±0.110  |
| Tyrosine                 | 2.74±0.032  | 4.32±0.054   | 4.34±0.062  |
| Phenylalanine + tyrosine | 7.68±0.064  | 10.00±0.121  | 9.94±0.148  |
| Threonine                | 3.24±0.041  | 3.82±0.059   | 3.04±0.042  |
| Tryptophan               | 1.38±0.036  | 1.26±0.028   | 1.24±0.033  |
| Valine                   | 5.98±0.082  | 5.51±0.064   | 4.27±0.072  |
| Alanine                  | 4.46±0.017  | 4.83±0.028   | 4.19±0.034  |
| Aspartic acid            | 11.25±0.084 | 11.46±0.099  | 11.82±0.114 |
| Glutamic acid            | 15.87±0.146 | 17.70±0.184  | 21.12±0.203 |
| Proline                  | 4.28±0.034  | 5.10±0.074   | 5.06±0.064  |
| Serine                   | 4.86±0.026  | 5.32±0.102   | 5.33±0.049  |
| Glycine                  | 3.85±0.048  | 4.63±0.067   | 6.30±0.102  |

\*Values represent means and their standard deviation for triplicate analysis of samples.

Table III summarizes the comparative protein quality of full-fat and defatted para-rubber seed meals, peanut and soybean meals and nutritional casein. The standard casein was better digested, supported significantly ( $p < 0.01$ ) superior body weight gains and showed significantly ( $p < 0.01$ ) superior protein quality indices compared to the other sources. Soybean meal was significantly ( $p < 0.05$ ) superior to peanut meal except for a similar true digestibility coefficient. Peanut meal was in turn superior to the rubber seed samples in terms of body weight gain and the protein quality indices measured. The full-fat rubber seed meal was significantly ( $p < 0.05$ ) better digested by rats, supported reasonably good gains and showed superior protein quality indices compared to the defatted sample, which did not support gain, thus giving negative PER values and low NPU and BV. The rat responses to diets containing either full-fat or defatted rubber seeds autoclaved for varying periods are presented in

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TABLE III. Comparative Nutritive Value of Full-fat and Defatted Rubber Seeds, Soybean, Peanut Meals and Nutritional Casein (Expt. I)

|                          | Para-rubber seed |           | Peanut meal | Soybean meal | Casein  | SE <sub>m</sub> |
|--------------------------|------------------|-----------|-------------|--------------|---------|-----------------|
|                          | Full-fat         | Defatted  |             |              |         |                 |
| Weight gain (14 days), g | 7.42 a           | -(8.66) b | 11.48 c     | 15.54 d      | 22.48 e | 0.56**          |
| Protein intake, g        | 8.83 a           | 6.71 b    | 7.46 bc     | 8.32 ac      | 8.23 ac | 0.47*           |
| PER                      | 0.84 a           | -(1.29) b | 1.54 c      | 1.88 c       | 2.73 d  | 0.16**          |
| NPU                      | 45.82 a          | 29.43 b   | 53.84 c     | 61.42 d      | 74.86 e | 1.48**          |
| BV                       | 49.87 a          | 38.51 b   | 59.42 c     | 65.38 d      | 79.86 e | 1.74**          |
| TD                       | 82.45 a          | 74.18 b   | 88.46 c     | 90.43 c      | 94.82 d | 1.31**          |

In this and subsequent tables the following indicate:

\* Significant treatment differences ( $p < 0.05$ )

\*\* Highly significant treatment differences ( $p < 0.01$ )

a, b, c ... treatment means followed by the same letters are not significantly different ( $p < 0.05$ ).

SE<sub>m</sub> = standard error of difference between treatment means.

Table IV. Autoclaving of both types of rubber seeds did not improve rat performance or protein quality indices. There was a tendency towards a worsening in PER, NPU and BV as well as TD as the autoclaving time increased. On average, the defatted meals also showed poorer responses compared to the full-fat meals.

Results of tests to evaluate the effect of extraction of full-fat and defatted rubber seed with either hot water, dilute hydrochloric acid or sodium hydroxide are summarized in Table V. Hot water extraction of both the full-fat and defatted rubber seeds improved weight gain, protein intake, PER, NPU and BV nonsignificantly in the case of the full-fat meals, but reduced significantly ( $p < 0.05$ ) the extent of weight loss in rats receiving the defatted samples. It improved PER and NPU but not BV and true digestibility. Dilute acid extraction significantly ( $p < 0.05$ ) depressed gains, and other protein quality indices except BV in rats receiving extracted full-fat rubber seeds. Similar depression in gains and protein quality indices were observed for the defatted meals except that only NPU and BV were significantly ( $p < 0.05$ ) lower than for the untreated. Sodium hydroxide extraction of both the full-fat and defatted rubber seed samples depressed gain, protein intake and all protein quality indices significantly compared to the untreated beans. In the case of the full-fat sample which had shown superior quality indices compared to the defatted body weight losses and very poor quality indices resulted.

Tables VI and VII summarize the results obtained when full-fat and defatted rubber seeds respectively were supplemented with

TABLE IV. Effect of Autoclaving for Varying Periods on the Protein Quality of Full-fat and Defatted Rubber Seeds (Expt. II)

| Treatment             | Autoclaving time (mins) | Weight gain (g)    | Protein intake (g) | PER                | NPU              | BV               | TD               |
|-----------------------|-------------------------|--------------------|--------------------|--------------------|------------------|------------------|------------------|
| Full-fat rubber seeds | 0                       | 6.43 a             | 8.41 a             | 0.77               | 43.42 a          | 50.87            | 83.44            |
|                       | 10                      | 5.49 ab            | 7.48 ab            | 0.73               | 42.98 a          | 50.46            | 82.78            |
|                       | 20                      | 4.87 b             | 6.96 b             | 0.70               | 37.84 b          | 48.32            | 81.63            |
|                       | 30                      | 4.91 ab            | 6.92 b             | 0.71               | 38.16 b          | 47.93            | 81.58            |
| Mean $\pm$ SE         |                         | 5.43 $\pm$ 0.52    | 7.44 $\pm$ 0.93    | 0.73 $\pm$ 0.14    | 40.60 $\pm$ 0.94 | 49.60 $\pm$ 0.94 | 82.36 $\pm$ 1.46 |
| Defatted rubber seeds | 0                       | -(10.23) a         | 8.06 a             | -(1.27) a          | 28.42 a          | 37.48            | 72.80 a          |
|                       | 10                      | -(7.48) b          | 7.63 a             | -(0.98) a          | 28.63 a          | 38.19            | 72.44 a          |
|                       | 20                      | -(8.03) b          | 6.24 b             | -(1.29) a          | 26.98 b          | 36.82            | 69.84 ab         |
|                       | 30                      | -(11.43) a         | 6.93 a             | -(1.65) b          | 24.83 c          | 36.34            | 66.87 b          |
| Mean $\pm$ SE         |                         | -(9.29) $\pm$ 0.48 | 7.22 $\pm$ 0.38    | -(1.30) $\pm$ 0.22 | 27.22 $\pm$ 0.49 | 37.21 $\pm$ 0.96 | 70.49 $\pm$ 1.33 |

Except for protein intake the means for all other parameters for the full-fat rubber seed meal were significantly ( $p < 0.01$ ) superior to the defatted meal.

TABLE V. Effect of Hot Water (HW) Dilute Acid (DA) and Dilute Sodium Hydroxide Extraction on the Nutritive Value of Full-fat and Defatted Rubber Seeds (Expt. III).

|          | Treatment     | Weight gain        |                 | Protein intake (g) | PER                | NPU              | BV               | TD               |
|----------|---------------|--------------------|-----------------|--------------------|--------------------|------------------|------------------|------------------|
|          |               | (g)                | (g)             |                    |                    |                  |                  |                  |
| Full-fat | None          | 6.87 a             | 8.68 a          |                    | 0.89 a             | 46.48 a          | 52.74 a          | 82.56 ac         |
|          | HW            | 7.50 a             | 8.11 a          |                    | 0.92 a             | 47.84 a          | 52.96 a          | 83.42 ac         |
|          | DA            | 4.24 b             | 7.42 a          |                    | 0.52 b             | 41.67 b          | 51.87 a          | 79.84 b          |
|          | SH            | -9.15 c            | 3.80 b          |                    | -2.65 c            | 21.31 c          | 28.38 b          | 80.45 bc         |
|          | Mean $\pm$ SE | 2.37 $\pm$ 0.64    | 6.75 $\pm$ 0.72 |                    | 0.35 $\pm$ 0.22    | 39.33 $\pm$ 1.84 | 46.49 $\pm$ 2.03 | 81.57 $\pm$ 1.76 |
| Defatted | None          | -(7.46) a          | 5.21 a          |                    | -(1.43) a          | 32.46 a          | 43.74 a          | 73.87 a          |
|          | HW            | -(3.48) b          | 5.38 a          |                    | -(0.65) b          | 38.98 b          | 46.30 a          | 74.80 a          |
|          | DA            | -(8.30) a          | 4.43 b          |                    | -(1.87) a          | 22.49 c          | 33.44 b          | 71.57 ab         |
|          | SH            | -(12.84) c         | 5.22 a          |                    | -(2.46) c          | 18.42 c          | 23.64 c          | 68.76 b          |
|          | Mean $\pm$ SE | -(8.02) $\pm$ 0.52 | 5.06 $\pm$ 0.48 |                    | -(1.60) $\pm$ 0.31 | 28.09 $\pm$ 1.46 | 36.78 $\pm$ 1.78 | 72.25 $\pm$ 1.43 |

\* Means for all parameters for the full-fat rubber seed meals were significantly ( $p < 0.05$ ) superior to the defatted meal.

TABLE VI. Effects of Amino Acid Supplements on the Nutritive Value of Full-fat Rubber Seeds (Expt. IV)

| Diet number     | Supplement (%)                                | Weight gain in 14 days | Protein intake | PER     | NPU      | BV       | TD       |
|-----------------|---|------------------------|----------------|---------|----------|----------|----------|
| 1               | None  | 4.72 a                 | 6.05 a         | 0.78 a  | 44.82 a  | 51.64 a  | 82.46    |
| 2               | 0.25 lys.                                     | 9.11 b                 | 6.95 a         | 1.31 b  | 53.63 bc | 62.49 bf | 83.42    |
| 3               | 0.15 meth.                                    | 9.95 b                 | 8.36 b         | 1.19 bc | 52.36 b  | 60.49 bd | 81.48    |
| 4               | 0.10 lleuc.                                   | 7.38 c                 | 8.58 b         | 0.86 ac | 46.83 ad | 53.32 a  | 82.44    |
| 5               | 0.25 lys.+0.15 meth.                          | 15.31 d                | 8.51 b         | 1.80 d  | 61.44 c  | 69.87 c  | 81.96    |
| 6               | 0.25 lys.+0.10 leuc.                          | 8.74 b                 | 7.11 ab        | 1.23 b  | 49.54 d  | 57.42 d  | 81.42    |
| 7               | 0.15 meth.+0.10 leuc.                         | 5.82 a                 | 6.33 a         | 0.92 ac | 47.58 ad | 56.84 d  | 82.36    |
| 8               | 0.25 lys.+0.15 meth.<br>+0.10 lleuc.          | 10.46 b                | 7.81 ab        | 1.34 b  | 55.48 ef | 63.82 bf | 82.44    |
| 9               | 0.25 lys.+0.15 meth.<br>+0.10 leuc.+0.05 try. | 11.42 b                | 7.42 ab        | 1.54 bd | 57.85 f  | 65.94 f  | 83.48    |
| SE <sub>m</sub> |   | 0.34**                 | 0.53*          | 0.18**  | 1.14**   | 1.48*    | 1.42(NS) |

TABLE VII. The Effect of Some Amino Acid Supplements on the Nutritive Value of Defatted Para-rubber Seed Meal- (Expt. IV)

| Diet number     | Supplement (%)                                 | Weight gain in 14 days | Protein intake | PER       | NPU     | BV       | TD       |
|-----------------|--|------------------------|----------------|-----------|---------|----------|----------|
| 1               | None   | -(3.42) a              | 4.69 a         | -(0.73) a | 28.44 a | 36.44 a  | 74.46    |
| 2               | 0.25 lys.                                      | 2.98 b                 | 5.74 ab        | 0.52 bd   | 42.46 b | 51.79 be | 73.82    |
| 3               | 0.15 meth.                                     | 1.82 c                 | 4.82 a         | 0.38 be   | 36.48 c | 46.83 c  | 74.24    |
| 4               | 0.10 lleuc                                     | -(0.27) d              | 5.55 ab        | -(0.05) c | 32.44 d | 41.64 d  | 72.86    |
| 5               | 0.25 lys+0.15 meth.                            | 4.39 e                 | 5.52 ab        | 0.80 d    | 46.16 e | 54.79 b  | 73.48    |
| 6               | 0.25 lys.+0.10 lleuc.                          | 3.56 c                 | 5.58 ab        | 0.64 d    | 40.95 b | 50.86 be | 73.92    |
| 7               | 0.15 meth.+0.10 lleuc.                         | 0.93 c                 | 6.15 b         | 0.15 e    | 37.68 c | 48.46 e  | 72.87    |
| 8               | 0.25 lys.+0.15 meth.<br>+0.10 lleuc.           | 4.06 e                 | 5.77 ab        | 0.70 d    | 43.68 b | 53.88 b  | 73.86    |
| 9               | 0.25 lys.+0.15 meth.<br>+0.10 lleuc.+0.05 try. | 3.34 e                 | 5.64 ab        | 0.59 d    | 40.87 b | 51.42 be | 71.98    |
| SE <sub>m</sub> |  | 0.37**                 | 0.42*          | 0.11**    | 1.04*   | 1.36*    | 1.72(NS) |

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amino acids. Supplementation of full-fat rubber seed with lysine or methionine significantly ( $p < 0.05$ ) improved gains, PER, NPU and BV, over and above those obtained for the basal unsupplemented diet. The improvement obtained with added methionine was less than obtained with lysine. Addition of isoleucine alone to full-fat rubber seed meal also significantly ( $p < 0.05$ ) improved gains but not PER, NPU and BV. The improvement in gain appeared to be related to a higher feed intake and therefore higher protein intake by rats on this diet compared to the basal. Combined addition of lysine and methionine produced significantly ( $p < 0.05$ ) the best gains, PER, NPU and BV and compared to the other diets. Addition of lysine and isoleucine or methionine and isoleucine produced inferior gains and protein quality indices compared to the diet with added lysine and methionine. Addition of tryptophan in the presence of the other three amino acids did not improve gain or protein quality indices any further. Responses of the defatted rubber seed meal to amino acid supplements were similar except that weight gain and protein quality indices were very much poorer compared to the responses of the full-fat meals.

### DISCUSSION

The compositional value presented for full-fat rubber seeds show protein and oil contents comparable to those reported for the more common oilseeds such as peanut, cashewnut and cottonseed (6,13) and suggest that used as the full-fat seed could be a promising high-protein-high energy source in animal feeds. The defatted meal however had a lower protein content compared to the levels reported for defatted peanut, cashewnut or cottonseed. Some compositional data exist from other sources on the various forms of rubber seed (1,2,3,4). The protein and oil contents reported for full-fat cake were slightly at variance with the one report (4) and more in line with some earlier analytical values (1). All the above reports in consonance with ours found low crude fibre levels in rubber seed, a fact which could be of considerable importance in the digestion and utilisation of associated nutrients. None of the earlier reports gave values for the mineral constituents of the rubber seeds. Our analysis indicates that this source could be a promising contributory source of mineral elements such as calcium, phosphorus, potassium, iron and zinc. Their relative availability however remains to be investigated. The amino acid data presented for rubber seed were similar to existing data except for slightly lower levels lysine, cystine, theanine and methionine in our samples compared to results presented in one earlier study (4). It is noteworthy however that in that study recovery of amino acids was reported to be enhanced by boiling fat extracted samples in 5% TCA before acid hydrolysis for total amino acid determination. Our results however suggest that low levels of lysine, methionine, tyrosine and marginal levels of isoleucine and phenylalanine could detract from the overall utilisation of rubber seed protein.

The biological evaluation of para-rubber seed showed this source to be poorer than either peanut or soybean meals. The wide differences in the value of rubber seeds and soybean may be expected from the observable clear differences in the level of some

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nutritionally important amino acids such as lysine, the sulphur amino acids and phenylalanine. The differences in the amino acid composition of rubber seeds and peanut meal were less marked. The later was however significantly better digested than the former and this digestibility differences could result in less availability of the already marginal or low levels of lysine and the sulphur amino acids manifesting itself in the observed poorer quality. More interesting is the marked difference in the protein quality of full-fat and defatted rubber seeds, the defatted samples showing an average difference of about 8 digestibility units compared to the full-fat samples and also being incapable of supporting growth in rats. The reasons for differences are not clear, but it is quite possible that the solvent used in oil extraction from rubber seeds may have altered the molecular conformation of the proteins resulting in altered solubility properties and therefore impairment of digestion. There are indications from studies with other oilseeds (14,15,16) that certain organic solvents minimise protein solubilization. The extent to which this occurred was shown to be related to the structure of the particular protein molecule and the presence of water within the seeds. The involvement of such solvent modifications in defatted rubber seed protein can only be speculative, since little or no information exists on its protein fraction make-up. It may be worth noting however, that collected seed were ground and extracted without further drying and contained a fair amount of moisture and this may have primed the kind of reactions speculated. On the other hand the greater digestibility of the full-fat meals could be attributable to the presence of fat within the cells which cause greater secretion of bile acids resulting in emulsification and exposure of greater surface area for enzyme penetration and attack and greater solubility and therefore enhanced digestion.

The results obtained from the autoclaving of both the defatted and full-fat samples are contrary to most observations with other oilseeds whose nutritive value have been shown to be improved by moist heat application within reasonable time limits. This probably suggests the absence of heat labile antinutritional factors in rubber seeds. One early report (3), had however suggested the presence of a cyanogenetic glucoside in some samples analysed which could yield prussic acid on hydrolysis. Depending on the nature of the glycoside bonding this could be heat stable. The presence of such a compound however which yield prussic acid, a compound whose detoxification in the animal body draws on the sulphur component of the sulphur amino acids (methionine and cystine) could result in an accentuation of the deficiency of these amino acids for productive purposes. Dilute acid and alkali extraction of both full-fat and defatted rubber seeds gave poor results, the alkali treatment being much worse than acid extraction. The most obvious explanation for this observed results may be a possible denaturation of the protein in the samples at the pH provided by the acid and alkali and the prolonged extraction period, resulting in decreased solubility, impaired digestion and decreased nitrogen absorbability. The slight improvements observed with hot water extraction could be due to the effects of solubilizing and leaching out of some toxic factors in the seed including prussic acid which has been suggested to be present (3). The beneficial effects of hot water extraction have been reported by several workers

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for rapeseed meal (17,18,19) and castor bean meal (20). The extent of improvement of any particular oilseeds must however be related to the concentration of water soluble toxicants that may be present in the material. It is quite possible that repeated extraction with water may have resulted in better results. This processing treatment needs therefore to be further investigated.

The amino acid supplementation results suggest that lysine and methionine are the amino acids most likely to limit the utilisation of rubber seed meals, thus suggesting that these seeds offer no advantage over the more commonly used oilseeds and oilseed cakes, the utilisation of most of which have been shown to be limited by low lysine and/or methionine contents (6,7,21). The differences in the responses of full-fat and defatted rubber seeds would appear to be related to the observed wide differences in digestibility. Impaired release of amino acids from the defatted meal, would tend to result in a more rapid absorption of the added amino acid supplement, which are made available faster than those from the native protein at sites of protein synthesis and may be subject to wasteful deamination with a resultant poor utilisation of the latter arriving amino acids. The faster solubilization and digestion of the full-fat samples would tend to provide a better balance of amino acids at any one time at utilisation sites.

The results presented are of a preliminary nature. The identification of the toxic or potentially toxic factors in rubber seeds, characterisation of the protein fractions could pave the way for more effective processing methods that will do minimum damage to the component nutrients, particularly the amino acids and thus help to maximise quality and optimize large scale and judicious use of available rubber seeds in livestock feeding.

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