EVALUATION OF NITROGEN UTILIZATION IN RATS FED MIXED DIETS OF BAMBARA GROUNDNUTS, RICE AND YAM

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ABSTRACT

Forty male albino rats were used to compare the nutritive value of mixed protein diets based on dehulled bambara groundnuts (DBG); undehulled bambara groundnuts (UDGB); rice flour (RF) and yam flour (YF) in young rats (45g-60g). These mixtures were nitrogenously formulated to provide 1.6g N/100g diet for 35 days. Combination of DBG: RF (80:20) and UDGB: RF (60:40) led to increase in both food and nitrogen intakes and PER compared to other mixtures (P < 0.05). Dehulling improved protein quality as judged by digested N and retained N, NPU, total plasma protein (TP) Dehulling also influenced weight gain and liver moisture. Replacement of an equal amount of RF with YF in the UDGB diet reduced intake, digested and retained N, BV, plasma protein, albumin, weight gain, PER, liver N, and moisture. The results of this study indicate that: Dehulling improves protein utilization, RF is superior to YF as substitute to BG regardless of N ratio, At lower ratio of 60:40 there appears to be a better EAA pattern as shown by increase in weight gain, PER.

Keywords: Nitrogen utilization, Rats, Mixed diets, Bambara Groundnuts, Rice, Yam

INTRODUCTION

Severe protein-energy malnutrition (PEM) remains a major health problem world wide, but more especially in the developing countries (Morlene, et al., 1997). PEM has for a long time plagued the developing countries. In these countries, about 30-40% of all children less than 5 years of age are malnourished. This phenomenon often leads to death (SCN, 1988; PIC, 1993). In most developing countries, more than half the population of weaning mothers cannot afford to buy milk, meat and other sources of first class animal protein because they are very expensive. As a result, infants may be weaned using predominantly starchy diets. Traditional weaning foods, where available, are not properly used because of lack of suitable food mixtures, parental ignorance or fallacies in the form of food prejudices and restriction.

Due to its high lysine content, the bean protein shows a beneficial complementary effect when consumed with cereal protein, which on the other hand contributes their methionine and cysteine to the infant formula. Infant weaning foods can be formulated on this basis (Orr, 1992). Research on the development of infant formula has long been going on in many laboratories on the evaluation of cereals supplemented with legumes, (Bolourichi et. al, 1968). Cowan and Pettet (1969) developed Laubina 106 as an infant food mixture for the Middle East. It is a mixture of wheat and legailts. Incaparina is a Guatemalan weaning food made from processed corn and cottonseed, flour, vitamins and minerals. Thripoosa is a weaning food made in Sirilanka. It is a mixture of corn and soyabeans. Other community level weaning formula include: weaning formula in Thailand prepared from rice, beans plus groundnut or sesame and Sarbottam Pitho made from roasted legumes, wheat and/or corn and/or rice in Nepal (SCN/ACC 2002). This study was therefore designed to determine the effectiveness of rice, Bambara groundnut and yam mixtures as complementary foods. The study also examined the effect of dehulling on the nutritive value of the diets and the ratio of these plant materials that will provide the best pattern of EAA for optimal growth in young rats. These young weaning rats are employed in this study for the following reasons: They require a greater level of protein than human infants for growth to take place. In other words, any level of protein in a weaning rat will support growth in a human infant. Another reason is that rats have shorter live span than humans and so will show effect of the test diets on growth faster than this will be shown in humans. We should also note that nutrition ethics demand that formulae still under test cannot be tested out first on humans but on test animals.

MATERIALS AND METHODS

Animal and Housing: Forty male albino-weaning rats were assigned to eight groups of five rats per group on the basis of their body weight. The difference in the weights of each group did not exceed 5g. The rats were weighed at weekly intervals to obtain changes in body weights. Feeding was done ad libitum. They were housed in individual metabolism cages made of steel and screen bottom, equipped to separate urine and faeces of the animals.

Preparation of Raw Material: All raw materials were purchased locally from Nsukka market. All the stones and dirt were removed from these materials before processing them differently to make them suitable for consumption. One half of the Bambara groundnuts was dehulled by first splitting the grains into halves using a dehulling machine. The split seeds were then soaked for about two hours to make dehulling easy. The rice was washed with clean water. The yam was peeled, washed, sliced into cubes and left in some saline liquid until time for boiling. The dehulled and undehulled Bambara groundnuts [DBG and UDGB respectively]; rice (RF) and yam (YF) were cooked until soft enough for consumption. These ingredients were then dried in an air oven for 1 hr, milled into fine flour using hammer mill (70 mesh) sieved and packaged into polythene bags pending use.

Diet Formulation: All food items were subjected to both proximate analysis and chemical analysis. Diet formulation was mainly based on results of the nitrogen analysis of the food materials and the dietary protein was 10%. Casein was used as the control diet. The distribution of the protein levels in the different diets is shown in Table 1.
Table 1: Diet Composition (in grams)

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<tr>
<td>Protein source in diet (%)</td>
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<tr>
<td>DBG:RF</td>
<td>80:20</td>
<td>799.9</td>
<td>0</td>
<td>0</td>
<td>558.5</td>
</tr>
<tr>
<td>DBG:RF:YF</td>
<td>80:10:10</td>
<td>799.9</td>
<td>0</td>
<td>382.21</td>
<td>279.25</td>
</tr>
<tr>
<td>UDBG:RF</td>
<td>80:20</td>
<td>0</td>
<td>074.5</td>
<td>0</td>
<td>58.5</td>
</tr>
<tr>
<td>DBG:RF:YF</td>
<td>80:10:10</td>
<td>0</td>
<td>974.5</td>
<td>279.2</td>
<td>282.21</td>
</tr>
<tr>
<td>DBG:RF</td>
<td>70:30</td>
<td>771.0</td>
<td>0</td>
<td>0</td>
<td>831.0</td>
</tr>
<tr>
<td>UDBG:RF</td>
<td>70:30</td>
<td>0</td>
<td>781.0</td>
<td>0</td>
<td>831.0</td>
</tr>
<tr>
<td>DBG:RF</td>
<td>60:40</td>
<td>661.0</td>
<td>0</td>
<td>0</td>
<td>1107.6</td>
</tr>
<tr>
<td>UDBG:RF</td>
<td>60:40</td>
<td>0</td>
<td>669.6</td>
<td>0</td>
<td>1107.6</td>
</tr>
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Table 2: Food Intake, Weight Gain, Per Liver Weight, Nitrogen and Moisture

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<tbody>
<tr>
<td>Food intake (g)</td>
<td>238.3</td>
<td>248.90</td>
<td>247.58</td>
<td>215.62</td>
<td>209.52</td>
<td>224.98</td>
<td>233.56</td>
<td>259.76</td>
<td>259.68</td>
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<tr>
<td>±0.28</td>
<td>±0.96</td>
<td>±1.46</td>
<td>±1.12</td>
<td>±0.91</td>
<td>±1.00</td>
<td>±1.50</td>
<td>±1.80</td>
<td>±1.30</td>
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<tr>
<td>Weight gain (g)</td>
<td>70.0</td>
<td>47.02</td>
<td>42.72</td>
<td>37.58</td>
<td>31.50</td>
<td>45.80</td>
<td>36.60</td>
<td>56.74</td>
<td>61.50</td>
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<tr>
<td>±0.28</td>
<td>±0.96</td>
<td>±1.46</td>
<td>±1.12</td>
<td>±0.91</td>
<td>±1.00</td>
<td>±1.50</td>
<td>±1.80</td>
<td>±1.30</td>
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<tr>
<td>Protein efficiency ratio</td>
<td>2.5</td>
<td>1.84</td>
<td>1.74</td>
<td>1.79</td>
<td>1.64</td>
<td>1.95</td>
<td>1.11</td>
<td>1.96</td>
<td>1.36</td>
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<tr>
<td>±0.28</td>
<td>±0.96</td>
<td>±1.46</td>
<td>±1.12</td>
<td>±0.91</td>
<td>±1.00</td>
<td>±1.50</td>
<td>±1.80</td>
<td>±1.30</td>
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<tr>
<td>Liver weight (g)</td>
<td>6.0</td>
<td>4.20</td>
<td>3.90</td>
<td>2.90</td>
<td>3.10</td>
<td>3.20</td>
<td>3.50</td>
<td>3.60</td>
<td>3.50</td>
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<tr>
<td>±0.80</td>
<td>±1.02</td>
<td>±1.43</td>
<td>±1.02</td>
<td>±1.02</td>
<td>±1.43</td>
<td>±1.02</td>
<td>±1.43</td>
<td>±1.02</td>
<td>±1.43</td>
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<tr>
<td>Liver nitrogen (g)</td>
<td>172.4</td>
<td>113.09</td>
<td>135.24</td>
<td>94.76</td>
<td>91.87</td>
<td>103.91</td>
<td>108.74</td>
<td>121.19</td>
<td>108.92</td>
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<tr>
<td>±59.50</td>
<td>±42.26</td>
<td>±39.47</td>
<td>±33.75</td>
<td>±25.99</td>
<td>±22.65</td>
<td>±29.21</td>
<td>±28.20</td>
<td>±35.67</td>
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<tr>
<td>Liver moisture (g)</td>
<td>57.76</td>
<td>55.04</td>
<td>57.12</td>
<td>47.04</td>
<td>58.66</td>
<td>48.18</td>
<td>43.30</td>
<td>41.49</td>
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<tr>
<td>±1.52</td>
<td>±7.02</td>
<td>±5.37</td>
<td>±16.53</td>
<td>±5.19</td>
<td>±9.37</td>
<td>±1.36</td>
<td>±1.74</td>
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<tr>
<td>Liver lipid (g)</td>
<td>36.00</td>
<td>34.00</td>
<td>34.00</td>
<td>36.00</td>
<td>37.20</td>
<td>22.00</td>
<td>19.00</td>
<td>29.00</td>
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<tr>
<td>±12.00</td>
<td>±3.74</td>
<td>±16.5</td>
<td>±14.28</td>
<td>±13.46</td>
<td>±6.00</td>
<td>±3.75</td>
<td>±5.83</td>
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Laboratory Analysis: The study period was 35 days out of which 28 days were for growth study and the other 7 days were for N balance study. Food intake was recorded every other day and the data were used for calculation of protein intakes of each animal during the N balance study. Food intake and body weight of the animals were recorded during the 28-day growth study period. The values were used to calculate protein efficiency ratio (PER).

Carmine red was fed on the morning of day 28 and on day 35. Colored faeces excreted on day 29 were included in the pooled faecal sample while those excreted on day 36 were excluded. Collection of urine started at 7:00 am of day 29 through the morning of day 36 and food consumption was measured for seven days. Hydrochloric acid (0.1N) was used to preserve the individual urine samples. The individual faeces collection were dried and weighed and then ground into fine powder. Diet, urine, faeces and their liver samples were assayed for N content by AOAC (1995) procedure. Moisture and fat content of the liver samples were determined at the end of the experiment. Blood proteins were assayed by the use of Ames blood analyzer.

Statistical Analysis: Means were separated using Duncan’s new multiple range tests (Steel and Torrie, 1960). This method was used to determine which dietary ingredient combinations were responsible for producing significant difference in parameters studied.

RESULTS

The crude protein content of the foods used as protein sources were as follows: RF 9.4%, DBG 23.25%, UDBG 21.55% and YF 6.85% (Table 1). The diets contained 10% protein (1.6g N/100g diet). Table 2 shows the mean for food intake, weight gain, protein efficiency ratio (PER); liver nitrogen weight, moisture and lipids for rats fed different mixed protein diets. Table 3 presents nitrogen intake, faecal, digested, urinary and retain nitrogen, biological value, net protein utilization and plasma protein of rats.

Food Intake: The 28-day food intake value for all groups varied between 259.8g for rats fed DBG: RF (60:40) and 209.5g for rats consuming UDBG: RF: YF (80:10:10) diet. However, there were no significant differences in food intake for rats fed different test diets (p > 0.05).

Growth: Growth increase was more pronounced in rats fed the control diet and least in animals fed DBG: RF: YF (80:10:10) diet. Animals fed UDBG: RF: YF (60:40) and DBG: RF (60:40) diets had weight gains similar to those fed the control diet (P < 0.05). The rest of the diets produced comparable weight gains, which were lower than for the above-mentioned diets (P < 0.05).
Nitrogen utilization in rats fed mixed diets of bambara groundnuts, rice and yam

Table 3: Nitrogen Intake, Fecal Nitrogen, Digested, Urinary and Retained Nitrogen; Biological Value, Net Protein Utilization, Total Protein (TP) Albumin and Globulin Values

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<tbody>
<tr>
<td>Nitrogen Intake (g)</td>
<td>±0.18</td>
<td>±0.70</td>
<td>±0.26</td>
<td>±0.16</td>
<td>±0.18</td>
<td>±0.09</td>
<td>±0.13</td>
<td>±0.88</td>
<td>±1.07</td>
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<tr>
<td>Faecal N (g)</td>
<td>0.08</td>
<td>0.19</td>
<td>0.18</td>
<td>0.21</td>
<td>0.23</td>
<td>0.20</td>
<td>0.18</td>
<td>0.19</td>
<td>0.26</td>
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<tr>
<td>Digested N (g)</td>
<td>±0.03</td>
<td>±0.08</td>
<td>±0.12</td>
<td>±0.08</td>
<td>±0.06</td>
<td>±0.06</td>
<td>±0.06</td>
<td>±0.06</td>
<td>±0.06</td>
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<tr>
<td>Urinary N (g)</td>
<td>±0.19</td>
<td>±0.07</td>
<td>±0.27</td>
<td>±0.16</td>
<td>±0.15</td>
<td>±0.14</td>
<td>±0.17</td>
<td>±0.15</td>
<td>±0.06</td>
</tr>
<tr>
<td>Retained N (g)</td>
<td>±0.08</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.01</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.02</td>
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<tr>
<td>BV</td>
<td>±0.18</td>
<td>±0.09</td>
<td>±0.27</td>
<td>±0.16</td>
<td>±0.14</td>
<td>±0.14</td>
<td>±0.14</td>
<td>±0.14</td>
<td>±0.07</td>
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<tr>
<td>Plasma (g/100 ml)</td>
<td>±0.08</td>
<td>±0.12</td>
<td>±0.08</td>
<td>±0.08</td>
<td>±0.10</td>
<td>±0.03</td>
<td>±0.07</td>
<td>±0.02</td>
<td>±0.02</td>
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<tr>
<td>Plasma Albumin (g/100 ml)</td>
<td>±0.6</td>
<td>±0.29</td>
<td>±0.26</td>
<td>±0.16</td>
<td>±0.14</td>
<td>±0.33</td>
<td>±0.21</td>
<td>±0.16</td>
<td>±0.32</td>
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<tr>
<td>Plasma Globulin (g/100 ml)</td>
<td>±0.7</td>
<td>±0.13</td>
<td>±0.12</td>
<td>±0.09</td>
<td>±0.07</td>
<td>±0.26</td>
<td>±0.09</td>
<td>±0.02</td>
<td>±0.16</td>
</tr>
<tr>
<td>Total Protein (g)</td>
<td>3.8</td>
<td>2.68</td>
<td>2.19</td>
<td>1.88</td>
<td>1.66</td>
<td>2.17</td>
<td>2.23</td>
<td>2.62</td>
<td>2.62</td>
</tr>
<tr>
<td>Nitrogen Loss (g)</td>
<td>±0.10</td>
<td>±0.17</td>
<td>±0.19</td>
<td>±0.09</td>
<td>±0.38</td>
<td>±0.13</td>
<td>±0.24</td>
<td>±0.72</td>
<td>±0.72</td>
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</table>

**Protein Efficiency Ratio (PER):** The PER values of all the groups varied. The rats fed UDBG: RF (70:30) mixture had the least PER value (1.11) while the animals fed the control diets had the highest PER (2.50). The rats fed DBG: RF (70:30), DBG: RF (60:40) and DBG: RF (80:20) had PER values comparable to the casein diet (1.84 and 1.96 respectively) but significantly higher than those of rats fed the rest of the diets (P < 0.05).

**Liver Weight (LW):** The LW for rats fed casein (6.0g) was significantly higher (P < 0.05) than for rats fed the rest of the diets (2.90 – 4.20g). The LWs for the rest of the diets were comparable.

**Liver Nitrogen (LN):** Liver N value (172.4g) for rats fed casein diet was highest (P < 0.05). The liver N value for rats fed DBG: RF: YF (80:10:10) diet was higher (135.24g) than for those animals fed the other test diets (p < 0.05). The rats fed UDBG: RF (80:20) and UDBG: RF: YF (80:10:10) had LN (94.76 and 91.87g respectively) that were significantly (P < 0.05) less than for those fed DBG: RF (80:20); DBG: RF (70:30); UDBG: RF (70:30); DBG: RF (60:40); UDBG: RF (60:40). The LW for rats fed casein (6.0g) was significantly higher (P < 0.05) than for those fed the rest of the diets (P < 0.05).

**Liver Moisture (LM):** The LM for rats fed UDBG: RF: YF (80:10:10), UDBG: RF (70:30), UDBG: RF (60:40) and UDBG: RF (60:40) were significantly lower (p < 0.05) than the LM for the rest of the rats fed the other diets. The highest value (37.20g) for liver lipid ( LL) was observed in rats fed DBG: RF (60:40) diet. This value was significantly higher than for those fed UDBG: RF (70:30) and DBG: RF (60:40) blends. The LL concentrations of rats fed other test diets were similar (p > 0.05).

**Food Nitrogen Intake (FNI):** The 7-day N balance study indicated that animals fed DBG: RF (60:40)(0.88g) and UDBG: RF: YF (80:10:10)(0.88g) diet had lower food nitrogen intake than those fed other diets. FNI of rats fed the control (1.0g) and DBG: RF (80:20)(1.10); DBG: RF: YF (80:10:10)(1.09g); DBG: RF (60:40)(1.07g) and UDBG: RF (160:40)(1.22g) were comparable but significantly higher than the FNI for rats fed the other diets.

**Digested Nitrogen (DN):** The DN value was higher (P < 0.05) for rats fed control, DBG: RF (80:20) and DBG: RF: YF (80:10:10) diets than for those fed UDBG: RF: YF (80:10:10) and UDBG: RF (70:30) mixtures. The DN values were similar for animals fed the other diets (P=0.05).

**Urinary Nitrogen (UN):** The UN of rats fed the control diet (0.12g) was significantly higher than those fed the test diets. The UN value (0.02-0.05g) for all the test groups was similar (P = 0.05).

**Retained Nitrogen (RN):** Rats fed DBG: RF (80:20) and DBG: RF: YF (80:10:10) diets had higher N retention value than those fed UDBG: RF (70:30) diets. The N retention values for all other groups were comparable (P=0.05).

**Biological Value (BV):** The BV values produced by rats fed all the diets were similar (P=0.05). There were no significant differences between the BV of rats fed the various diets.

**Net Protein Utilization:** There were no significant differences in the NPU value for both control and test groups (P > 0.05). The NPU values ranged from 0.72-0.81.

**Total Plasma Protein (TPP):** The animals fed the control diet, DBG: RF (80:20), DBG: RF (60:40) and UDBG: RF (60:40) had total plasma protein values that were significantly higher than those of animals fed the rest of the diets. The albumin levels of the rats in the different groups varied significantly (P < 0.05). Rats fed the control diet produced the highest values (3.89g). The DBG: RF (60:40) diets had higher albumin value compared to the other groups while the least value was produced by those fed UDBG: RF: YF (80:10:10) diet. There were significant differences in globulin values for rats fed all test diets.
Nitrogen utilization in rats fed mixed diets

except those fed of UDBG: RF: YF (80:10:10) and DBG: RF (70:30) diets (p > 0.05).

DISCUSSION

In this work, dehulling of the Bambara groundnuts was beneficial to the fed rats. The higher weight gain values for rats fed the control diet are not surprising. It shows that a low intake of good quality protein provides desirable patterns of EAA, which the animal would use to synthesize body tissue protein than those animals consuming higher food intake of low quality protein. The result suggests that the weight gain of the rats fed the different diets agreed with the food intake of the rats. The higher food intake of rats fed UDBG: RF (60:40) and DBG: RF (60:40) agreed with their higher weight gain. This shows that their diets provide desirable pattern of EAA equal to those of casein. Other works have observed that all vegetable protein diets at certain ratios could match animal protein in providing EAA suitable for growth. (Morlene et al 1997).

Increase in ratios of legume and cereal improves PER values of diets. The higher PER values of UDBG: RF (60:40) suggests the production of a better pattern of essential amino acid (EAA) by this diet. This finding is closely related to the work of Ketiku and Ladoye (1984) who reported that the best PER values were obtained from a 50-50 ratio of rice and beans. The liver N, moisture and lipid values suggest that dehulling improves antinutritional factors and exposes the protein to the action of proteolytic enzymes thereby supplying equal EAA used in synthesis of liver proteins. The impact of good protein nutrition is especially marked in liver cells (Orr; 1992).

The lower N digestibility of rats fed UDBG lead to high faecal N excretion which may be attributed to high fiber in the diets, a phenomenon commonly observed in all vegetable-rich diets (Uauy and Yanez, 1998). There were very slight variations in BV values for all groups of rats. The RN for all groups of rats was influenced by food nitrogen intake and faecal/urinary nitrogen output. The comparable RN of some of the diets when compared with casein suggests they have EAA pattern close to those of animal proteins. The higher BV values for all test groups when compared with casein may be due to some analytical errors, which sometimes are associated with N balance studies. This is because animal proteins are expected to have higher BV than plant proteins. Even though there were no significant differences in the NPU values for both control and test groups (p > 0.05), dehulling conferred higher NPU to the diets.

The lower plasma albumen levels of rats fed UDBG: RF: YF (80:10:10) appear to indicate that the replacement of an equal amount of RF with YF did not supply enough EAA in the liver of the animals for the synthesizing of albumin. Albumin is synthesized in the liver and depends solely on the dietary sources. Once the amino acids are not supplied, the albumin concentration decreases, hence albumin is a very sensitive measure of protein quality. These results prove that vegetable protein when well processed and combined can provide EAA pattern that will equal that of animals. Uauy and Yanez (1983) fed Chilean children a mixture containing wheat-31%, potatoes-15%, rice-10% and beans-19%. When compared to those fed whole dried egg protein, there were no significant differences between the N intakes of both diets.

Conclusion: These results confirm that plants represent valuable and economic sources of edible protein especially when appropriate varieties and processing technologies are used. In this study, dehulling had a positive effect on the protein quality of the diets while replacement of RF with an equal amount of YF in DBG–containing diets improved the protein quality of the diets. However, these diets would still have to be tested on human subjects before a conclusion can be drawn on the adequacy of these mixtures for infant feeding.

REFERENCES