

**THE EVALUATION OF THE PROTEIN QUALITY OF
SOYBEAN PRODUCTS BY SHORT-TERM BIOASSAYS IN
ADULT HUMAN SUBJECTS^{1,2}**

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SUMMARY

The protein quality of soybean products was determined by a short nitrogen balance index (NBI) assay with 15 adult males. In the first study, a textured vegetable protein (TVP) was tested alone and in a 50:50 mixture with ground beef. Protein was fed at 0.2, 0.4 and 0.6 g/kg body weight/day with a constant energy intake. The protein quality (NBI) was then calculated by regression analysis of nitrogen absorbed to nitrogen retained. The NBI of beef (0.91) and of the mixture (0.87) were similar; however, the

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protein quality of TVP fed alone (0.77) was significantly lower. In the second study, carried out at an intake level of 0.6 g protein/kg/day, supplementation of the TVP with 0.5% DL-methionine improved nitrogen retention, but the values obtained did not reach statistical significance. Meat alone, or the 50:50 mixture fed at an equivalent protein intake, gave similar nitrogen retention values, both of which differed significantly from those obtained when TVP was fed alone. The addition of wheat gluten, used as a source of methionine, to the TVP: meat mixture, produced nitrogen retention values below those obtained with the 50:50 beef:TVP blend.

Ten men participated in another trial, this time to determine the protein quality of a soybean protein isolate, which gave an NBI value of 0.91, indicating the high quality of the protein tested. Comparison of the protein quality values obtained by the short-term NBI assay revealed these were essentially the same as those reported by other workers using the conventional long-term approach.

INTRODUCTION

Soybean protein as flours, concentrates, or isolates is beginning to be utilized in a variety of food systems for populations in developed and developing countries. In order to retain the nutritional properties of the foods where they are used, availability of chemical and biological assays becomes necessary to monitor their quality and that of the products made from them. Bressani *et al.* (1) investigated the protein quality of a texturized food simulating ground beef made from isolated soybean protein with added egg albumin and wheat gluten, in experimental animals and children. It was found that 138 mgⁱ of nitrogen/kg body weight/day were required from the soybean texturized food, as compared with 97 mg from milk to maintain nitrogen equilibrium in young children. Kies and Fox (2) compared the protein nutritional value of TVP, methionine-enriched TVP, and beef, at two levels of protein intake for the adult man. The higher protein level of 8.0 g nitrogen/day fed to each subject was capable of maintaining all of them in nitrogen equilibrium regardless of the source of protein, whereas the 4.0 g nitrogen intake induced a negative nitrogen balance in each one of the subjects. At this level of protein intake there was a clear response to the addition of methionine. More recently, Kies and Fox (3) have presented data on the effect of varying the ratio of beef and texturized vegetable protein on the nitrogen balance of human adults.

A better quality protein was observed as more meat replaced the TVP, suggested by an increase in nitrogen retention. Zezulka and Calloway (4) found that nitrogen retention in men fed a soy protein isolate improved with the addition of methionine, even when the level of intake was as low as 3 g protein nitrogen. The same experimental subjects were fed 4.5 and 6.0 protein nitrogen from egg white. At these levels of intake, nitrogen retention was similar to that obtained with comparable levels of protein nitrogen from a soybean isolate supplemented with methionine. The protein quality of soybean and soybean products for children and young adult human subjects has been recently reviewed by Bressani (5) and by Young and Scrimshaw (6). The biological methodology utilized in these studies was the nitrogen balance method, either at a single point of intake or using multiple points of nitrogen intake. In this last approach, both the quality of the protein and the amount of protein to be ingested for nitrogen equilibrium are obtained. Although this long-term approach is highly acceptable, it has the inconvenience of the long time needed to obtain protein quality values. To eliminate some of these problems, a short-term multiple point assay was proposed and its applicability has already been tested for egg and milk proteins (7, 8).

In the present study, the protein quality of various protein products was assessed using the short-term balance method in young adult human subjects, and the values obtained were compared to published values on similar products using the conventional approach.

MATERIALS AND METHODS

Two soybean products were utilized. One was a TVP product manufactured in Guatemala and the second a protein isolate.⁴ The TVP was tested alone and in a 50:50 mixture with ground beef.

With TVP, studies were also performed to test the effect of methionine addition as well as of wheat gluten, tested as a source of methionine.

The short-term NBI method described in previous publications (7, 8) was used to evaluate the protein quality of these

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products. The assay itself consisted in feeding a basal low nitrogen-free diet prepared with a low-nitrogen wheat starch bread, starch cookies and a few low-nitrogen fruits and vegetables. This diet provided between 21.1 to 27.0 mg nitrogen/kg/day. Carbonated beverages, jelly and hard candy were added to maintain body weight. Meal preparation and details of menus were the same as described in a previous article (8).

All subjects were given a daily vitamin supplement. Each tablet contained vitamin A (synthetic), 1.5 mg; vitamin D, 12.5 mcg; thiamine mononitrate, 10 mg; riboflavin, 10 mg; sodium ascorbate, 300 mg; niacin, 100 mg; piridoxine hydrochloride, 2 mg; calcium pantothenate, 20 mg; vitamin B₁₂, 4 mcg; copper sulfate, 3.92 mg; iron sulfate, 31.3 mg; potassium iodine, 0.196 mg; calcium carbonate, 125 mg; manganous sulfate, 3.08 mg; magnesium sulfate, 29.7 mg and potassium sulfate, 11.142 mg.

Enough beef was purchased from a local market. The sirloin was trimmed from fat and tendons, ground twice and mixed well before weighing out individual portions. These were wrapped, stored in plastic bags and frozen for later use.

Each portion of meat, TVP, or the combination of both, was cooked individually in pyrex dishes with cottonseed oil, onion and tomato sauce, and then eaten with cornstarch spaghetti or wheat-starch bread. Wheat gluten was added to the sauce when needed; the methionine was given with drinking water.

Quantitative urine and feces collection were made every two days.

TVP Studies

Fifteen subjects participated in these studies, divided into three groups with an average weight of 58 kg per group. Their physical characteristics are presented in Table 1.

Group I received soybean (TVP); Group II received meat, and Group III the 50:50 protein mixture from TVP and meat. For three consecutive days the subjects consumed only the basal diet so as to determine endogenous nitrogen losses, followed by feeding 0.2, 0.4 and 0.6 g protein/kg/day each for two days. The first phase of the experiment lasted nine days; two more days on the last protein level were studied, during which Group I was fed soybean (TVP) plus 0.5% DL-methionine. Group II continued on meat, and Group III received 50% protein from meat, 25% from soybean (TVP) and 25% from wheat gluten. The purpose

TABLE 1

**PHYSICAL CHARACTERISTICS OF THE SUBJECTS
FED SOYBEAN (TVP) AND MEAT**

| | Subject | Weight kg | Height cm | Caloric intake Kcal/kg |
|----------|---------|------------------|------------------|---------------------------|
| TVP | HM | 52 | 163 | 50 |
| | AM | 54 | 168 | 45 |
| | EM | 57 | 162 | 45 |
| | PM | 61 | 166 | 45 |
| | JE | 64 | 179 | 44 |
| | | 57.6 ± 0.09 | 167.6 ± 0.04 | 45.8 ± 0.05 |
| Meat | FS | 51 | 167 | 45 |
| | MM | 55 | 164 | 45 |
| | WH | 59 | 172 | 45 |
| | RC | 59 | 170 | 45 |
| | HG | 66 | 168 | 43 |
| | | 58.0 ± 0.10 | 168.2 ± 0.02 | 44.6 ± 0.02 |
| TVP:Meat | AF | 52 | 171 | 45 |
| | RR | 55 | 172 | 45 |
| | AG | 56 | 169 | 45 |
| | RE | 59 | 168 | 45 |
| | AC | 66 | 166 | 43 |
| | | 57.6 ± 0.09 | 169.2 ± 0.01 | 44.6 ± 0.02 |
| | | 57.73 ± 0.08 | 168.3 ± 0.03 | 45.0 ± 0.03 |

was to observe if nitrogen balance would improve after achieving positive retention with the 0.6 g intake, by the addition of the limiting amino acid methionine, or complementation with a cereal protein.

Soybean Protein Isolate

Evaluation of the soybean protein isolate was carried out in 10 young adult subjects whose physical characteristics are detailed in Table 2. As in the case of the TVP studies, the subjects were first fed a nitrogen-free diet previously described (7, 8) for three days, followed by feeding 0.2, 0.4 and 0.6 g protein/kg body weight/day, each for a two-day period. The protein isolate was served as cream of tomato soup, replacing the milk in the soup.

The individual and group regressions of N intake to N balance and N absorbed to N balance were calculated for both studies.

TABLE 2

PHYSICAL CHARACTERISTICS OF THE SUBJECTS FED
A COMMERCIAL PROTEIN ISOLATE*

| Subject | Weight kg. | Height cm | Caloric intake Kcal/kg |
|---------|-----------------|------------------|---------------------------|
| SAA | 57.1 | 163 | 43 |
| GM | 57.5 | 165 | 43 |
| MM | 57.5 | 157 | 43 |
| RR | 58.0 | 171 | 45 |
| PM | 61.2 | 167 | 43 |
| AG | 58.0 | 168 | 43 |
| AL | 57.5 | 161 | 43 |
| RA | 58.0 | 168 | 43 |
| MER | 57.1 | 166 | 43 |
| AC | 55.7 | 170 | 43 |
| | 57.8 ± 0.46 | 165.6 ± 1.42 | 43.2 ± 0.21 |

* Ralston Purina Co., St. Louis, Mo., USA.

RESULTS

Table 3 presents data on the average nitrogen balance of the experiments carried out with TVP alone and mixed with beef. A positive retention by all individuals in the group was observed with the 0.6 g protein intake. The highest retention occurred when ground meat was fed alone, followed by those fed the 50:50 mixture and the TVP by itself. On the average, the group fed TVP or meat alone reached positive balance at the 0.4 g protein intake level. The values for the nitrogen balance index, that is, the regression of N absorbed to N balance, are presented in Figure 1 and were 0.77, 0.87 and 0.91 for the TVP, meat and 50:50 mixture, respectively. These values indicate that N equilibrium calculated from the regression equations was obtained with N absorbed from 64, 70 and 68 mg/kg/day of meat, meat:TVP and TVP, in that order. The regressions of N intake to N balance shown in Table 4 indicate that zero nitrogen balance was obtained when the intake was 83, 91, and 95 mg/kg/day for meat, meat:TVP and TVP, respectively.

Data on nitrogen balance during the second phase of the experiment are shown in Table 5. These indicate that the supplementation of TVP with methionine increased nitrogen retention; however, it did not reach statistical significance. The retention values found for meat alone or for the 50:50 mixture were essentially the same and higher than those derived from TVP alone, the difference being statistically significant. The addition of wheat gluten to the TVP:meat mixture resulted in N retention values below those observed with the 50:50 beef:TVP blend.

Table 6 summarizes the nitrogen balance data of the studies carried out with the soybean protein isolate. In this case, positive nitrogen balance was reached on the average at an intake of 0.4 g protein/kg/day, but some subjects were still negative at this level. The individual and pooled regression equations between N intake and N retention and between N absorbed and N retention are shown in Table 7. Average nitrogen intake for nitrogen equilibrium was 85, and for N absorbed and zero balance, 62 mg/kg/day, values which fall between those found for soybean protein as presented in this report.

DISCUSSION

Evaluation of the protein quality of soybean TVP in young adult human subjects, fed either alone or mixed with meat.

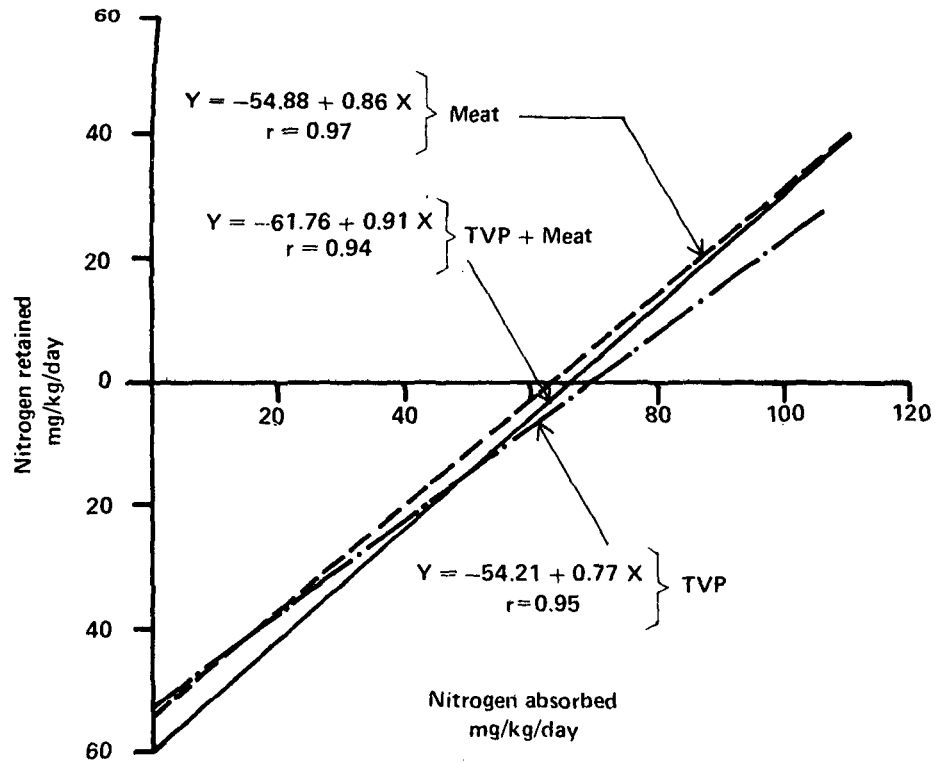


FIGURE 1
 Nitrogen balance index of soybean protein (TVP) and meat

TABLE 3

AVERAGE APPARENT NITROGEN BALANCE IN HUMAN ADULTS FED SOYBEAN PROTEIN (TVP), MEAT, AND TVP:MEAT COMBINATIONS

| | Protein level g/kg | Intake | Absorbed mg/kg/day | Retained |
|----------|-----------------------|-------------|-----------------------|-------------|
| TVP | 0.0 | 26.6 ± 0.9 | 5.6 ± 2.7 | -56.0 ± 2.6 |
| | 0.2 | 52.2 ± 0.8 | 35.0 ± 1.6 | -22.0 ± 5.8 |
| | 0.4 | 86.6 ± 0.9 | 63.2 ± 4.0 | 0.0 ± 1.4 |
| | 0.6 | 131.4 ± 1.4 | 102.0 ± 3.1 | 19.8 ± 4.8 |
| Meat | 0.0 | 27.0 ± 1.1 | 3.0 ± 1.5 | -56.0 ± 2.7 |
| | 0.2 | 52.6 ± 0.8 | 31.6 ± 2.2 | -23.4 ± 4.7 |
| | 0.4 | 87.0 ± 1.0 | 67.0 ± 4.6 | 5.8 ± 7.4 |
| | 0.6 | 131.8 ± 1.4 | 110.4 ± 1.0 | 37.8 ± 2.1 |
| TVP:Meat | 0.0 | 26.8 ± 1.0 | -1.0 ± 2.4 | -69.8 ± 9.4 |
| | 0.2 | 52.6 ± 0.8 | 37.6 ± 2.4 | -15.4 ± 4.0 |
| | 0.4 | 87.0 ± 1.0 | 62.0 ± 3.9 | - 6.6 ± 3.2 |
| | 0.6 | 131.6 ± 1.4 | 106.2 ± 2.7 | 33.0 ± 3.7 |

using nitrogen balance has been reported by other workers (5, 6, 9). Kies and Fox, for example (2, 3), found that when TVP was fed at the suboptimal level of 4 g N/day, nitrogen balance was negative, and that the addition of methionine improved its quality. At this low level of intake (4 g N/day) higher retention values were obtained with meat, suggesting, therefore, that TVP is inferior in quality when fed at low intake levels. When intake was increased to 8 g N/day, nitrogen retention values were similar to those reported for meat. In another study, Korslund, Kies and Fox (10) compared the nitrogen retention of adolescent boys fed textured vegetable protein (TVP) with and without 1% DL-methionine, compared to that obtained with beef. The 4.0 g N/day were able to support positive nitrogen retention in the subjects fed the fortified TVP or the beef, but not when the TVP was fed without fortification. Based on this experience and on another from the same group of investigators, the authors suggested fortification of the textured soy product, especially if it is used alone.

TABLE 4

REGRESSION EQUATIONS BETWEEN NITROGEN INTAKE AND
NITROGEN BALANCE

| Intake = X | | Standard error | |
|-----------------|------------------------|----------------|------|
| Retention = Y | | | |
| <i>Meat</i> | | | |
| 11C | $Y = -66.46 + 0.87 X$ | 0.96 | 0.17 |
| 12C | $Y = -84.55 + 0.87 X$ | 0.98 | 0.11 |
| 13C | $Y = -80.27 + 0.93 X$ | 0.98 | 0.11 |
| 14C | $Y = -60.90 + 0.79 X$ | 0.96 | 0.16 |
| 15C | $Y = -79.18 + 0.88 X$ | 0.95 | 0.06 |
| | $Y = -74.41 + 0.88 X$ | 0.95 | 0.06 |
| <i>TVP</i> | | | |
| 1S | $Y = -73.02 + 0.76 X$ | 0.96 | 0.14 |
| 2S | $Y = -65.40 + 0.58 X$ | 0.93 | 0.16 |
| 3S | $Y = -54.92 + 0.58 X$ | 0.95 | 0.13 |
| 4S | $Y = -61.95 + 0.75 X$ | 0.91 | 0.24 |
| 5S | $Y = -74.36 + 0.79 X$ | 0.98 | 0.09 |
| | $Y = -65.73 + 0.69 X$ | 0.92 | 0.07 |
| <i>TVP:Meat</i> | | | |
| 6T | $Y = -98.15 + 0.97 X$ | 0.91 | 0.31 |
| 7T | $Y = -104.90 + 1.03 X$ | 0.93 | 0.28 |
| 8T | $Y = -71.98 + 0.81 X$ | 0.96 | 0.16 |
| 9T | $Y = -58.38 + 0.78 X$ | 0.93 | 0.21 |
| 10T | $Y = -70.63 + 0.83 X$ | 0.98 | 0.11 |
| | $Y = -79.90 + 0.87 X$ | 0.90 | 0.10 |

TABLE 5

NITROGEN BALANCE OF YOUNG MEN FED MEAT, SOYBEAN (TVP) AND ITS COMBINATION
WITH THE ADDITION OF METHIONINE OR WHEAT GLUTEN

| Protein level | | Intake | Urine | Fecal | Absorption | Retention |
|---------------------|-------------|-------------|------------|------------|-------------|------------|
| | | mg N/kg/day | | | | |
| Meat | 0.6 | 131.8 ± 1.4 | 72.6 ± 2.6 | 21.4 ± 0.5 | 110.4 ± 1.0 | 37.8 ± 2.1 |
| | 0.6 | 129.8 ± 1.4 | 73.2 ± 5.2 | 23.2 ± 3.4 | 106.6 ± 3.9 | 33.2 ± 6.3 |
| Soybean (TVP) | 0.6 | 131.4 ± 1.2 | 82.6 ± 4.4 | 29.4 ± 4.2 | 102.0 ± 3.1 | 19.8 ± 4.8 |
| | 0.6 + meth. | 129.8 ± 1.2 | 71.2 ± 5.1 | 30.8 ± 4.3 | 99.0 ± 4.8 | 28.0 ± 5.5 |
| TVP + meat | 0.6 | 131.6 ± 1.4 | 73.2 ± 4.6 | 25.4 ± 2.1 | 106.2 ± 2.7 | 33.0 ± 3.7 |
| TVP + meat + gluten | 0.6 | 129.8 ± 1.3 | 87.4 ± 6.7 | 19.0 ± 2.3 | 110.8 ± 3.1 | 23.2 ± 6.9 |

TABLE 6

NITROGEN BALANCE OF MEN FED A SOYBEAN PROTEIN ISOLATE*

| Protein level g | Intake | Absorbed | Retained |
|--------------------|--------------|-------------|--------------|
| | mg N/kg/day | | |
| 0.0 | 21.1 ± 0.18 | 0.5 ± 1.70 | -61.3 ± 3.37 |
| 0.2 | 50.1 ± 0.10 | 27.8 ± 1.86 | -27.8 ± 4.35 |
| 0.4 | 83.0 ± 0.01 | 60.7 ± 2.74 | 6.3 ± 5.16 |
| 0.6 | 125.2 ± 0.10 | 95.1 ± 4.4 | 26.1 ± 5.02 |

* See Table 2.

TABLE 7

REGRESSION EQUATIONS BETWEEN NITROGEN INTAKE
AND NITROGEN BALANCE OF SUBJECTS FED SOYBEAN
PROTEIN ISOLATE

| Intake = X Retention = Y | | r | Standard error B |
|-----------------------------|---------------------|------|---------------------|
| 1SAA | Y = -63.13 + 0.83 X | 0.94 | 0.21 |
| 2 GM | Y = -64.48 + 0.85 X | 0.96 | 0.15 |
| 3MM | Y = -74.48 + 0.86 X | 0.94 | 0.20 |
| 4RR | Y = -71.59 + 0.74 X | 0.99 | 0.03 |
| 5PM | Y = -69.86 + 1.06 X | 0.98 | 0.15 |
| 6AG | Y = -65.68 + 0.75 X | 0.97 | 0.11 |
| 7AL | Y = -84.69 + 0.88 X | 0.95 | 0.18 |
| 8RA | Y = -64.38 + 0.70 X | 0.89 | 0.24 |
| 9MR | Y = -99.37 + 0.88 X | 0.97 | 0.13 |
| 10AC | Y = -70.64 + 0.87 | 0.91 | 0.27 |
| | Y = -72.20 + 0.84 X | 0.90 | 0.06 |

it is shown that methionine supplementation of the local product improved its quality when fed at 0.5% DL-methionine. The results obtained from the soybean protein isolate study suggest this product to be of high quality. From the values presented, an intake of 86 mg N/kg/day of the isolate are needed for N equilibrium, which is similar to the calculated value for meat as reported in this paper. Recently, Young and Scrimshaw (6) and Scrimshaw and Young (9), using a conventional multiple point nitrogen balance assay of long duration, found that the protein isolate in question produced nitrogen equilibrium when fed at the level of 0.67 g protein/kg/day. This value, which includes losses of nitrogen, compares well with that obtained from the study presented, which was 0.54 g protein/kg/day. The same authors report an intake of 0.59 and 0.64 g protein/kg/day for a 50:50 beef:TVP and beef, respectively, while according to the results of the present study, these values correspond to 0.57 and 0.53 g protein/kg/day, respectively. These results confirm the quality of the proteins under study and support the validity of the short-term multiple point nitrogen balance assay, applied already to egg and milk protein (7, 8).

As indicated before (8), the short-term approach gives slightly higher values, tending to slightly overestimate the protein quality. This effect, which is presently under study, may be due to two possible factors in methodology, as already suggested (7, 8). One is the 3-day depletion period on the nitrogen-free diet, which in future studies will be decreased, and the second, that the method does not allow an adaptation period before balance. In any event, and recognizing that the proteins used in our study were not tested by the conventional long-term assay, the method is sensitive enough, since the values obtained confirm results reported by other investigators using the conventional approach. Furthermore, because of its short duration, it is of low cost and permits the evaluation of variables affecting the multiple point assay.

RESUMEN

EVALUACION DE LA CALIDAD PROTEINICA DE PRODUCTOS DE SOYA POR MEDIO DE ENSAYOS BIOLÓGICOS DE CORTA DURACION EN HUMANOS

Se determinó la calidad proteínica de productos de soya por

medio de un ensayo de índice de balance nitrogenado (NBI) con 15 individuos adultos del sexo masculino. En el primer estudio se sometió a prueba una proteína vegetal texturizada (TVP), sola y en una combinación de 50:50 con carne de res. La proteína se administró a niveles de 0.2, 0.4 y 0.6 g/kg peso corporal/día, respectivamente, con una ingestión constante de energía. La calidad proteínica (NBI) se calculó por análisis de regresión del nitrógeno absorbido al nitrógeno retenido. Los NBI de la carne de res (0.91) y de la mezcla de proteína de soya y carne de res (0.87) fueron muy similares; sin embargo, la calidad proteínica de la TVP sola (0.77) fue significativamente menor. En un segundo estudio y con un nivel de ingestión de 0.6 g proteína/kg/día, la suplementación de la TVP con 0.5% DL-metionina mejoró la retención de nitrógeno, pero los valores obtenidos no alcanzaron significancia estadística. Con la carne de res sola o en una combinación de 50:50 con TVP, administradas a niveles equivalentes de proteína, se obtuvieron valores similares de retención de nitrógeno, significativamente diferentes de los resultantes de la administración de TVP sola. La adición de gluten de trigo como fuente de metionina a la mezcla carne de res:TVP produjo valores de retención de nitrógeno por debajo de los obtenidos con la mezcla carne de res:TVP.

En otro ensayo, que esta vez incluyó 10 individuos adultos del sexo masculino, se determinó la calidad proteínica de un aislado de soya; éste arrojó un valor de NBI de 0.91, lo que indica la alta calidad del producto. Comparaciones realizadas entre los valores de calidad proteínica obtenidos con el ensayo de NBI de corta duración mostraron que éstos eran esencialmente iguales a los obtenidos por otros investigadores aplicando el método convencional de larga duración.

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