Nutritional Assessment of Two Vegetable Protein Concentrates in Growing Rats

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The digestive and metabolic utilizations of vegetable protein concentrates were determined in growing rats with the nutritional balance method of Thomas–Mitchell. The test diets contained 12% protein in the form of casein plus 5% DL-methionine (control), commercial potato (PPC) and wheat (WPC) concentrates, and supplemented wheat protein concentrate (sWPC). The chemical composition of the protein sources was also determined to detect possible deficiencies in essential amino acids in PPC and WPC. The aminogram for PPC showed the content in amino acids to be adequate for the requirements of growing rats. The WPC presented a low content in tyrosine and some essential amino acids (methionine, tryptophan, and cysteine). The PPC had excellent nutritional value, as was indicated by the indices protein efficiency ratio (PER), apparent (ADC) and true digestibility coefficients (TDC) for protein, biological value (BV), net protein utilization (NPU), and nitrogen retention (nitrogen balance), which reflect the nutritive use of protein. However, the digestive and metabolic utilizations of WPC were poor and did not improve significantly after supplementation with amino acids. This poor response may have been due to the industrial production process which could have affected the bioavailability of some essential amino acids.

INTRODUCTION

One of the most important lines of research in animal nutrition is devoted to the search for new, economical raw materials for feeds. Intensive animal husbandry requires the development of feeds that avoid excessive dependence on a single ingredient or on a few ingredients.

The need for new sources of dietary protein has been stimulated by the rapid consumption by humans of traditional sources such as fish and meat. The formulation of a feed containing new ingredients for a given species requires detailed chemical analyses as well as biological assessments in rats or some other standard laboratory animal.

Vegetable protein concentrates are among the potential sources of protein for commercial feeds. Two examples that have recently become available are potato and soluble wheat protein concentrates. The results published to date regarding the former are inconclusive, although successes have been reported with potato protein concentrate as a replacement for soy protein or powdered milk in feeds designed for pig fattening (Eeckhout, 1980; Urbanczyck et al., 1983) and as a partial substitute in mink (Charlet-Lery et al., 1985) and broiler chicken feeds (Helder and Versteegh, 1977). The results with potato protein concentrate as a partial substitute for fish flour protein in eel (Hidalgo, 1988) and trout feed (Moyano, 1990) have been poor. Wheat protein constitutes a subproduct when starch is extracted from wheat; it has a favorable emulsifying quality which leads to its use as an alimentary ingredient in the production of commercial feed products (meat-based products, sauces, fats, etc.).

The purpose of the present study was to characterize the potential nutritional value of potato and soluble wheat protein ingredients in animal nutrition. Our findings should provide animal nutritionists with practical information on additional sources of nutrients and could foreseeably help to alleviate temporary shortages in raw materials by extending the choice of ingredients.

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Table I. Analytical Composition of the Control Protein and Protein Concentrates in Dry Matter

	casein, %	PPC, %	WPC, %
crude protein	90.32	84.63	86.79
protein nitrogen content	87.87	87.72	91.65
moisture	8.10	8.93	3.82
fat	1.3	1.05	6.00
ash	2.8	2.63	3.66
carbohydrates		12.42	4.00

MATERIALS AND METHODS

Two vegetable proteins were assayed according to the Thomas–Mitchell method (Mitchell, 1923): potato protein concentrate (Lysamine, France) and wheat protein concentrate (Amypro SWP, Campo del Ebro Industrial, Zaragoza, Spain).

A series of four experiments were performed. In control analyses, dietary protein consisted of casein plus 5% DL-methionine. In the other three experiments, the source of dietary protein was potato protein concentrate (PPC), wheat protein concentrate (WPC), or supplemented WPC (sWPC). The analytical composition of the diets is shown in Table I.

Diet and bidistilled water were provided *ad libitum*, and the experiments were performed in two phases. During the initial period, dietary protein (casein plus 5% DL-methionine) was adjusted to 4%, an amount considered sufficient to record depletion. Three days were allowed for the animals to adapt to the diet and experimental conditions, and data were recorded during the following 3 days. During the main experimental period (following 10 days), dietary protein content was adjusted to 12% in all experiments. Three days were allowed for adaptation to the experimental diet, and 7 days were used to record data. Throughout the initial and experimental periods, body weight, food intake, and output of feces and urine were recorded. In all, five diets were tested (Table II).

In each experiment 10 young albino Wistar rats were used (5 male, 5 female). The growing animals (age 21 days at the start of the experiment) were housed in individual metabolic cages,

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Table II. Analytical Composition of Diets (Percent Dry Matter)

	endogenus	control	PPC	WPC	sWPC
Theoretical Composition					
casein	4.00	12.00			
PPC			12.00		
WPC				12.00	12.00
DL-Met + Cys					2.00
tryptophan					0.80
fat (olive oil)	4.00	4.00	4.00	4.00	4.00
mineral suppl[a]	4.00	4.00	4.00	4.00	4.00
vitamin suppl[a]	1.00	1.00	1.00	1.00	1.00
fiber (cellulose)	8.00	8.00	8.00	8.00	8.00
sugar and starch in equal parts to make up 100% of dry weight					
Analytical Composition					
nitrogen	0.59	1.69	1.82	1.87	1.73
protein	3.72	10.54	11.39	11.67	10.83
fat (olive oil)	4.20	5.07	5.10	5.44	5.38
fiber (cellulose)	8.00	8.00	8.00	8.00	8.00
moisture	6.18	5.16	5.97	5.62	5.16
ash	3.39	3.59	4.02	4.33	3.33
NFEM[b]	21.92	73.95	71.70	71.07	73.57

[a] Commonwealth Bureau of Nutrition (1979). [b] Nitrogen-free extractive material.

which were kept in a thermoregulated room (22 ± 1 °C) with a controlled 12-h light/dark period (lights on at 9:00 a.m.).

The following indices and parameters were determined for each group: intake (expressed as dry weight), body weight, protein efficiency ratio (PER); apparent (ADC) and true digestibility coefficients (TDC) for protein; biological value (BV); net protein utilization (NPU); and nitrogen retention (nitrogen balance).

$$\text{PER} = \frac{\text{weight gained (g per rat per day)}}{\text{protein intake (g per rat per day)}} \quad (1)$$

$$\text{ADC} = \frac{I - F}{I} \times 100 \quad (2)$$

$$\text{TDC} = \frac{I - (F - F_k)}{I} \times 100 \quad (3)$$

$$\text{BV} = \frac{I - [(F - F_k) - (U - U_k)]}{I - (F - F_k)} \times 100 \quad (4)$$

$$\text{NPU} = \frac{I - [(F - F_k) - (U - U_k)]}{I} \times 100 \quad (5)$$

$$\text{balance} = I - (F + U) \quad (6)$$

In accordance with the formulas recommended by the FAO/OMS (1966), the factors used were I (nitrogen intake), F (fecal nitrogen), F_k (endogenous fecal nitrogen), U (urinary nitrogen), and U_k (endogenous urinary nitrogen).

Body weight and protein intakes were expressed as grams per rat per day.

Analytical Techniques. Nitrogen was determined according to the Kjeldahl method; the protein conversion factor was 6.25.

Water content was determined by oven-drying at 105 ± 1 °C until a constant weight was obtained.

Protein nitrogen content was determined in protein precipitated with copper acetate.

The amino acid composition of the proteins was determined by high-performance liquid chromatography (Pico-Tag method) of acid-digested samples (except for tryptophan); cysteine and methionine were analyzed after performic acid oxidation.

Ether extraction was performed by gravimetry of the ethyl ether extract.

Ash was measured by calcination at 500 °C to a constant weight.

The results from all experiments and analyses were tested statistically by analysis of variance with the ONEWAY procedure of the SPSS/PC software package. Means were compared with Duncan's test.

RESULTS

Chemical Analyses. The amino acid composition of the protein concentrates tested and that of the control are shown in Table III. It was found that WPC was deficient in methionine, tryptophan, and cysteine and PPC low in cysteine, on the basis of the nutritional requirements of the growing rat (National Research Council, 1978).

Table III. Amino Acid (AA) Composition of Potato Protein (PPC), Wheat Protein (WPC), and Casein plus 5% DL-Methione (Grams per 100 g of Protein)

AA	PPC	WPC	casein
Asp	10.22	17.38	06.35
Glu	09.67	13.89	19.36
Ser	04.45	06.30	05.05
Gly	04.65	05.89	01.80
His	02.44	03.29	03.44
Thr	04.82	06.63	05.42
Ala	04.45	05.75	02.69
Arg	03.34	04.40	03.21
Pro	06.24	05.27	06.35
Tyr	05.01	00.06	04.93
Val	06.38	07.00	05.59
Ile	03.48	03.83	04.56
Leu	07.64	08.11	08.67
Phe	05.69	06.31	04.63
Lys	07.58	07.51	07.17
Met	03.76	01.39	07.99
Trp	01.40	01.00	02.17
Cys	00.89	02.07	00.54
total branched AA	17.50	18.94	18.82
total sulfur-containing AA	04.65	03.46	08.72
total aromatic AA	10.70	06.37	11.72

Table IV. Chemical Score in the Protein Concentrates

amino acid, %	PPC, %	WPC, %	amino acid, %	PPC, %	WPC, %
phenylalanine	98.10	108.79	methionine	121.29	44.88
tyrosine	119.28	1.43	cysteine	37.08	86.85
isoleucine	52.73	88.33	valine	87.40	95.89
leucine	86.82	92.16	tryptophan	87.50	62.50
lysine	118.44	117.34			

The percent relative amounts of essential amino acids in dietary proteins are presented in Table IV referenced to chicken egg albumin.

Table I gives the values for percent protein in casein and experimental concentrates studied.

Biological Analyses. Daily food intake was significantly greater for animals fed control (casein) and WPC diets ($p < 0.001$) than for those given PPC and sWPC. When these results were expressed as grams of food per 100 g body weight, however, the only significant difference was the greater intake of the PPC group compared to that of the WPC ($p < 0.001$) group (Table V).

Daily weight increases were significantly greater in the control and PPC rats in comparison with those fed the other two diets. When body weight was calculated as grams increase per grams of protein consumed (PER), we found that increases in body weight were significantly greater after feeding with casein and PPC than with WPC and sWPC ($p < 0.001$) (Table V).

Nitrogen absorption with the different diets is shown in Table VI. The digestive utilization of nitrogen was significantly higher in the two groups that consumed non-wheat-derived sources of dietary protein ($p < 0.001$). The ADC of protein was greater in rats fed potato or wheat protein concentrate than in the control group ($p < 0.001$). The ADC in the PPC group, although elevated, was significantly lower than that in the control group ($p < 0.008$), whereas ADC in grops WPC and sWPC were higher than in the control group; the difference, however, was significantly only for rats fed WPC ($p < 0.001$). The TDC approached 100% in all groups, with no significant differences between the diets.

In addition to having lower food intakes, the rats in

Table V. Food Intake and Weight Change in Rats Fed Diets Containing Different Types of Protein

group	mean body weight, g	dry daily matter intake g/rat	dry daily matter intake g/100 g of rat	daily protein intake, g/rat	PER[a]
control	100.21 ± 3.23	12.66 ± 0.49 bc	12.63 ± 0.27	1.33 ± 0.05	3.26 ± 0.21 bc
PPC	94.54 ± 1.58	13.13 ± 0.29 de	13.90 ± 0.27 d	1.50 ± 0.03	2.90 ± 0.11 de
WPC	76.04 ± 2.20	8.58 ± 0.43	11.32 ± 0.54	1.00 ± 0.05	0.84 ± 0.11
sWPC	71.20 ± 1.91	8.86 ± 0.47	12.41 ± 0.47	0.96 ± 0.05	0.84 ± 0.08

[a] PER = weight gained (g/rat/day)/protein intake (g/rat/day).

Table VI. Digestive Utilization

group	daily nitrogen intake, mg/rat	daily total fecal nitrogen, mg/rat	daily endogenous fecal nitrogen, mg/rat	daily absorbed nitrogen, mg/rat	ADC	TDC
control	213.52 ± 8.24	20.70 ± 1.17	14.60 ± 0.79	207.42 ± 8.97	90.15 ± 0.71	96.98 ± 0.63
PPC	239.12 ± 5.21	30.93 ± 2.12	14.87 ± 0.68	223.06 ± 4.46	87.12 ± 0.72	93.33 ± 0.63
WPC	160.20 ± 8.11	11.10 ± 0.57	9.04 ± 0.43	158.13 ± 7.94	93.04 ± 0.20	98.73 ± 0.27
sWPC	153.53 ± 8.15	12.49 ± 0.70	14.60 ± 0.79	155.64 ± 8.34	91.68 ± 0.64	97.57 ± 0.71

Table VII. Metabolic Utilization[a]

group	daily total urinary, mg/rat	daily endogenous urinary, mg/rat	daily food nitrogen, mg/rat	biological value	net protein	balance
control	63.48 ± 5.13	31.25 ± 1.42	32.23 ± 4.32	84.48 ± 1.97 b2c2	81.97 ± 2.17 b2c2	129.34 ± 7.95 b2c2
PPC	75.69 ± 3.81	29.75 ± 1.45	45.94 ± 2.84	79.47 ± 1.11	74.16 ± 1.12	132.49 ± 3.13
WPC	109.42 ± 5.30 b2d2	20.85 ± 1.02	88.57 ± 5.14	44.14 ± 1.06	43.59 ± 1.11	39.68 ± 2.57
sWPC	109.52 ± 6.34 c2e2	31.25 ± 1.42	78.26 ± 5.94	49.98 ± 1.95	53.12 ± 1.90	31.53 ± 5.40

[a] Letters and numbers following values indicate the following: a, significant difference between control and PPC; b, significant difference between control and WPC; c, significant difference between control and sWPC; d, significant difference between PPC and WPC; e, significant difference between PPC and sWPC; 1, $p < 0.01$; 2, $p < 0.001$.

groups WPC and sWPC showed significantly higher urinary excretion rates than the animals fed casein or PPC ($p < 0.001$) (Table VII).

Biological values were similarly elevated in the control and PPC groups, whereas this indicator was significantly lower in rats that consumed wheat-derived protein ($p < 0.001$) in comparison with controls, the absolute values being lower by approximately half.

Net protein utilization showed similar increases in the control and PPC groups and was significantly lower than the control value in groups WPC and sWPC ($p < 0.001$).

Nitrogen balance was similar after feeding with the control diet and PPC. However, the balance was significantly lower (by approximately two-thirds) after feeding with diet WPC ($p < 0.001$). Diet sWPC produced no significant improvement in nitrogen balance in comparison with the control value.

DISCUSSION

Chemical Analyses of Protein Nutrition. Amino acid content in PPC was, in general, adequate to cover the requirements of growing rats. The composition we report is similar to those found in earlier studies (Hanczakowski and Skraba, 1989) for different sources for potato protein concentrates.

The proportion of essential amino acids of approximately 50% in PPC and WPC makes both concentrates of moderately good nutritional value. According to the reference values given in a review by Santidrian (1987), proteins of high nutritional value consist of approximately 33% essential and 66% nonessential amino acids, while those of low nutritional value contain 25% essential and 75% nonessential amino acids.

The essential/nonessential amino acid ratio (E/N ratio) was approximately equal to 1 in both PPC and WPC, making both sources of protein appropriate for growing rats, according to the findings of Stucki and Harper (1962). These researchers reported optimal growth after weaning with feeds in which the E/N ratio was 4:1.

These findings suggest that good growth would be predicted in young rats fed either of the vegetable protein concentrates we tested. However, WPC did not lead to good growth, probably because the analytical methods used did not take amino acid imbalances or differences in their rates of absorption into account. The results of biological analyses were more informative on this point (see below).

The chemical scores of PCC and WPC were 37.08 and 44.84, respectively; these values were influenced by the low cysteine and methionine, respectively, values. Despite the low chemical scores, we refrained from supplementing PPC with cysteine, because of the good digestive and metabolic utilization of this protein. Also, this protein satisfies the cysteine plus methionine requirements of growing rats (National Research Council, 1978).

In contrast, because WPC did not cover these requirements, we tested it after supplementation with methionine, tryptophan, and cysteine, the three most limiting amino acids, according to the chemical score. Because previous studies have reported contradictory results with regard to the usefulness of supplementation with the first limiting amino acid only (Byers, 1983; Ohshima, 1985), we supplemented WPC with amounts of the first three limiting amino acids as required for rats.

Percent protein in the sample was calculated as 85% and 87%, respectively, for PPC and WPC (Table I); these values confirm the high purity and quality of these two products in comparison with others described in the literature (Telek, 1983; Kahlon and Arora, 1986).

Protein nitrogen content, or true protein content, indicated that nearly all nitrogen levels in the three sources of dietary protein tested were protein, with a low proportion of free amino acids.

The similarities between the control protein (casein), PPC, and WPC in percent protein content and protein nitrogen facilitated comparisons of the metabolic and digestive parameters.

Biological Analyses of Protein Content. Food intake results during the main experimental period showed that WPC was not well accepted by the rats. The amino acid

imbalance was the most likely cause of the poor results obtained with this source of protein. Other authors (Leung and Rogers, 1969) noted that diets deficient in amino acids, particularly in essential amino acids, markedly decreased food intake. In this connection, WPC is deficient in tyrosine and tryptophan. The supplemented WPC we tested was also deficient in tyrosine, which was not added because it is not an essential amino acid for rats. The low tyrosine content together with the low bioavailability of some essential amino acids due to the industrial process (this is discussed later on) may partly account for the decreased food intake in the sWPC group, in agreement with the findings of Li and Anderson (1983). Further studies will be needed to confirm this connection.

The good intakes of the PCC-containing diet suggest that the concentration of solanine (400 ppm) did not affect the palatability to rats (Moyano, 1990), in contrast with the findings in fish by this author. A more accurate indication of intake is given by the results expressed as grams of food consumed per 100 g body weight (Montellano et al., 1983; Lopez Frias et al., 1985). When intake was expressed as a function of weight, the results were similar for control, WPC, and sWPC diets, although supplementation of the WPC diet increased intake slightly. This finding concurs with the observations of Kwong and Barnes (1977), who tested diets containing 12% casein or wheat gluten and found slightly higher intakes when cystine was added to the low-casein diet and lysine or lysine plus threonine to the wheat gluten diet.

Protein efficiency ratios (PER) were similar in rats fed the control and PPC diets but were markedly lower in animals that consumed WPC when compared with those given casein as the dietary source of protein. However, our values in the WPC group were similar to those reported by Andersson et al. (1989) for a wheat-containing diet given under experimental conditions similar to ours. Harper et al. (1970) noted that diets containing inadequate proportions of amino acids led to both decreased intake and growth alterations. Supplementation of WPC did not improve PER.

The proportion of total fecal nitrogen to both nitrogen intake and absorbed nitrogen was similar, at approximately 10% with casein, 13% with PPC, and 7% with WPC and sWPC. With reference to fecal nitrogen loss in the control group, the greater food intake in the PPC group led to a proportional increase in fecal loss, whereas in the WPC and sWPC groups, the lower intakes were compensated for by the correspondingly lower rates of fecal excretion, which probably reflected the organism's attempt to conserve ingested nitrogen.

As a consequence of these results, the ADC of all three protein concentrates tested was elevated, being similar to that found for casein. Although the PPC diet yielded the lowest ADC in the present study, nitrogen absorption was nonetheless highly efficient. The high solanine content in this protein concentrate apparently had no effect on digestibility, as was found earlier with intake. This may have been because orally administered solanine is poorly absorbed in the gastrointestinal tract and is hydrolyzed in the stomach to solanidine, a less toxic substance (Dalvi and Bowie, 1983).

Fecal loss of nitrogen tended to decrease as the rats in groups WPC and sWPC consumed less feed; consequently, ADC values were higher (albeit not significantly so) than those of the casein group. Those findings suggested that although the imbalanced amino acid composition of WPC reduced food intake, this apparently had little effect on intestinal absorption of this protein concentrate.

The metabolic utilization of PPC, assessed as BV, was as high as that found in the control group. The factors that contributed to the favorable BV were high intake and values of total and endogenous urinary exretion similar to those in control animals. Under our experimental conditions, BV of PCC was nearly 80%, a figure much higher than the 62% BV reported by Hanczakowski and Skraba (1989). This emphasizes the good nutritional quality of the PPC tested in the present study.

However, BV is a limited indicator, in that it reflects the amount of absorbed nitrogen only. We therefore calculated the NPU of PPC and found it to be similar to the NPU in the control group.

The relatively high solanine content of PPC not only had no effect on intake or digestive utilization but also appeared to have no detrimental effect on the nutritional value of this source of protein. This was probably due to the rats being resistant to this alkaloid (Gull, 1961).

The BV of WPC was significantly lower than that of casein, owing to the lower intake and greater urinary excretion in rats given wheat-derived protein. The reduction in endogenous urinary nitrogen also may have contributed to the lower BV in this group.

Supplemented WPC led to a slight improvement in BV, which nonetheless remained significantly lower than that obtained in control animals, probably because of limited improvement in food intake and urinary nitrogen excretion, which remained similar to those found after feeding with unsupplemented WPC. In a study of growing chickens, Terapuntuwat and Tasaki (1984a,b) found that supplementation of vegetable protein concentrate with deficient amino acids failed to improve the nutritive utilization of the concentrate in comparison with the control feed.

The industrial production of protein concentrates for foods and feeds usually involves heat treatment with alkalines or acids, which may affect the amino acid balance, especially lysine. Heating to high temperatures decreases the BV by favoring the formation of peptide bonds between lysine side chains and dicarboxylic acids. Moreover, acid or basic treatment reduces tryptophan and lysine (Cowey, 1979). The method used to produce WPC appears to have markedly altered its protein structure, to the detriment of metabolic utilization, which was not improved by the addition of supplementary amino acids. We should note that we may not have calculated supplementation correctly, owing to the low bioavailability of some of the amino acids in WPC; this factor was pointed out previously for other vegetable protein concentrates (Terapuntuwat and Tasaki, 1986). The presence in wheat of several endogenous antinutritional substances (Tacon and Jackson, 1985; Haraszti and Vetter, 1986), which may not have been completely neutralized in the process of producing WPC, may also have curtailed the metabolic utilization of this source of protein.

The main conclusions from this study are the following:

The chemical evaluation of wheat (supplemented) and potato protein concentrates assessed in relation to the aminogram, chemical score, crude protein, and protein nitrogen content indicates that both protein concentrates have an adequate content of amino acids which cover the requirements for growing rats.

Wheat protein concentrate contains a low-quality protein in terms of biological value for growing rats. Supplementation with the three limiting amino acids does not improve the nutritive utilization, possibly due to the changes that the protein undergoes during the industrial production process.

Potato protein concentrate contains a high-quality protein, as shown in the apparent (ADC) and true digestibility coefficients (TDC) for protein, biological value (BV), not protein utilization (NPU), and nitrogen retention (nitrogen balance) indices, whose values indicate high absorption and metabolic utilization which lead to optimum growth levels.

Further studies are needed to test the bioavailability of amino acids.

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