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Nutritional Evaluation of Pea (*Pisum sativum* L.) Protein Diets after Mild Hydrothermal Treatment and with and without Added Phytase

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The effect of mild hydrothermal treatment and the addition of phytase under optimal conditions (pH 5.5, 37 °C) on the nutritive utilization of the protein of pea (*Pisum sativum* L.) flour was studied in growing rats by examining the chemical and biological balance. Mild hydrothermal treatment produced reductions of 83, 78, and 72%, respectively, in the levels of α-galactosides, phytic acid, and trypsin inhibitors and also produced a significant increase in the digestive utilization of protein. The additional fall in the levels of phytic acid caused by the addition of phytase did not lead to a subsequent improvement in the digestive utilization of protein. The mild hydrothermal treatment of pea flour produced a significant increase in the metabolic utilization of protein and carbohydrates, which was reflected in the protein efficiency ratio and food transformation growth indices. These effects were not observed in the phytase-supplemented pea diet.

KEYWORDS: ***Pisum sativum* L.; phytase; phytic acid; mild hydrothermal treatment; nutritive utilization; protein**

INTRODUCTION

Legume seeds are an important source of dietary protein, carbohydrates, minerals, vitamins, and antioxidants, with great potential for human and animal nutrition. The nutritive utilization of legumes can be negatively affected by their content of antinutritional factors such as α-galactosides, trypsin inhibitors, or phytic acid, which interfere with the ingestion and digestive utilization of protein and minerals by monogastric animals.

The detrimental effect of phytic acid on protein digestibility arises from its ability to interact with protein, forming two different complexes, depending on the pH (*1*). Binary protein−phytate complexes are formed at acidic pH, and ternary protein−mineral−phytate complexes are formed via a cationic bridge as the pH approaches neutrality. The reduced solubility of proteins as a result of protein−phytate complexes can also adversely affect certain functional proteins, the activity of which depends on their hydration and solubility (*2*). The possibility that phytate may inhibit proteolysis by inhibition of digestive proteinases has been suggested by Singh and Krikorian (*3*), but subsequent investigations have failed to demonstrate this (*4*).

In recent years, the widespread use of phytase in animal nutrition, intended to improve the nutritive utilization of phytate phosphorus utilization and to decrease the environmental pollution caused by undigested phosphorus in effluents from swine and poultry units (*5*, *6*), has provided new insights into the antinutritive properties of phytate in relation to protein utilization. Selle et al. (*7*) pointed out the importance of the relative solubility of phytate salts and proteins from different feed ingredients and their effects on the extent of protein−phytate complex formation, coupled with variations in the effectiveness of phytase in different dietary matrices, as important factors in the effect of phytate and phytase on the nutritive utilization of protein. Nevertheless, information about the effect of phytase on the content of other nutrients and antinutritional factors is scarce.

There are some reports on phytase supplementation to foods for human consumption (*8*−*10*), but most of these concern phosphorus or iron availability and do not consider protein. This paper reports a study of the nutritive utilization of protein from peas to which suitable concentrations of phytase were added in order to minimize the antinutritional properties of phytic acid with regard to the digestive and metabolic utilization of protein.

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MATERIALS AND METHODS

Diets. *Raw Pea Flour (RP). Pisum sativum* L. var. Esla was from the germplasm collection of Valladolid (Spain).

Mild Hydrothermal Treatment without Phytase Addition (PNP). Raw pea flour was incubated in 0.1 N acetic/sodium hydroxide buffer, pH 5.5, at 37 °C for 60 min in a stirring bath with a speed of 350 rpm. The ratio of flour to soaking solution was 1:10 (w/v). After the incubation, the mixture was centrifuged at 15317*g* and the supernatant discarded. The flour was then frozen and freeze-dried.

Mild Hydrothermal Treatment with the Addition of Phytase (PP). Raw pea flour was incubated in 0.1 N acetic/sodium hydroxide buffer, pH 5.5, at 37 °C for 60 min in a stirring bath with a speed of 350 rpm and treated with 800 units of phytase/kg of feed (*Aspergillus niger* phytase, Novo Nordisk, Denmark). One unit of phytase activity is defined as the amount of enzyme that liberates 1 μmol of inorganic phosphorus from sodium phytate per minute at pH 5.5 and 37 °C. The rest of the procedures applied were the same as for the PNP diet. All of the experimental diets were supplemented with 5% olive oil prior to being fed to the animals.

Analyses. The moisture content of the different pea diets was determined by drying to constant weight in an oven at 105 ± 1 °C. Total nitrogen was determined according to Kjeldahl's method. Crude protein was calculated as N × 6.25. Protein and non-protein nitrogen were measured using the methodology described by Periago et al. (*11*).

The pH of raw and processed peas was determined after 5 g of the sample was resuspended in 40 mL of distilled water with a Crison GLP22 pH meter (Crison, Barcelona, Spain). The samples were then titrated with 0.1 M NaOH during agitation to pH 7. Titrable acidity was expressed as milliequivalents of NaOH per 100 g of dry matter (DM).

Determination of Available Soluble Sugars and α-Galactosides. Analysis of glucose, fructose, sucrose, and α-galactosides (raffinose, ciceritol, stachyose, and verbascose) was carried out following the method described by Frías et al. (*12*).

Determination of Vitamins B_1 and B_2. A single extraction procedure for vitamins B_1 and B_2 was carried out according to that of Vidal-Valverde et al. (*13*). These vitamins were quantified by HPLC as described previously (*13*, *14*).

Trypsin Inhibitor Activity (TIA) Determination. TIA was determined as described in Vidal-Valverde et al. (*13*).

Phytic Acid Determination. Inositol hexaphosphate (IP_6) was determined by HPLC according to the method of Kozlowska et al. (*15*).

Biological Methods. *Experimental Design and Diet.* We used a biological balance technique, recording changes in body weight and food intake and then calculating nitrogen intake and fecal and urinary nitrogen excretion. Three 10-day experiments, in which raw or processed peas were the only food source, were carried out. During the first 3 days of experiments, the rats were allowed to adapt to the diet and experimental conditions, and the main experimental period comprised the next 7 days, during which body weight and food intake were recorded and feces and urine were collected for analysis.

Animals. In each experiment we used 10 young albino Wistar rats (5 males and 5 females). The growing animals (recently weaned), with an initial body weight of 111 ± 1.6 g, were housed from day 0 of the experiment in individual stainless steel metabolic cages designed for the separate collection of feces and urine; the cages were located in a room with a 12 h light/dark period, at a temperature of 21 ± 2 °C, fitted with an appropriate ventilation system. Throughout the experimental period all rats had free access to double-distilled water, and the diet was consumed ad libitum. At the end of the experimental period the animals were anesthetized with CO_2 and killed by decapitation. The liver and the longissimus dorsi muscle were collected for analysis. The rats were handled at all times in accordance with current European regulations regarding laboratory animals.

Biological Indices. The following indices and parameters were determined for each group according to the formulas given below: intake (expressed as dry weight), body weight gain, protein efficiency ratio (PER; weight gain in grams per day/protein intake in grams per day); food transformation index (FTI; total intake in grams of dry matter per day/increase in body weight in grams per rat per day); apparent digestibility coefficient (ADC) (i); nitrogen retention (nitrogen balance) (ii); and percent nitrogen retention/nitrogen absorption (% R/A) (iii):

$$\mathrm{ADC} = [(I - F)/I] \times 100 \quad \text{(i)}$$

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

$$\%\ \mathrm{R/A} = \{[I - (F + U)]/(I - F)\} \times 100 \quad \text{(iii)}$$

where I = intake, F = fecal excretion, and U = urinary excretion.

Statistics. Data were subjected to multifactor analysis of variance using Statgraphics Statistical Graphics 5.0 System software (Statistical Graphics Corp., Rockville, MD).

RESULTS

Chemical Analysis. Table 1 summarizes the content of nutrients and the antinutritional factors of raw pea flour and the flour obtained after mild hydrothermal treatment at 37 °C and pH 5.5 with or without the addition of the phytase enzyme. When no enzyme was added, only endogenous phytase would have acted during the mild hydrothermal treatment. As a result of the treatment, the nitrogen content fell significantly, by 6% in the control group (no added phytase). This reduction was mainly due to the decrease in non-protein nitrogen. In the group given the phytase-supplemented diet, the fall in nitrogen content was 7%, mainly due to the decrease in protein nitrogen. The pH in the processed diets fell significantly, from 6.5 in raw peas to 5.6 in the control diet with no added phytase and to 5.7 in the phytase-supplemented diet. There was an increase in the titratable acidity of the diets, from 34 mequiv of $NaOH \cdot kg^{-1}$ of DM in the raw-pea flour diet to 95.4 and 94 mequiv of $NaOH \cdot kg^{-1}$ of DM in the control (non-phytase) diet and the phytase-supplemented diet, respectively.

Significant increases were observed in processed peas in total available sugars (11−12%) and total and available starch (12−17%), whereas there was a significant decrease in total available soluble sugars (57−78%) and vitamin B_1 (40%) and B_2 (70%) content. The content of α-galactosides fell by 83−84%, and TIA decreased sharply from a level of 8.7 in raw peas to 2.45 and 2.2 when pea flour was subjected to mild hydrothermal treatment with or without phytase enzyme addition, respectively.

The phytic acid content of the pea flour decreased significantly after mild hydrothermal treatment without phytase (78%) and was further reduced by the addition of phytase (93%).

Biological Analysis. Food intake, expressed in grams/rat/day or per 100 g of body weight/day, was similar in all of the tests carried out (**Table 2**). No significant differences were caused by the treatment applied to the pea flour. These results, together with those concerning the chemical composition of the protein, the available starch, available soluble sugars, and vitamins B_1 and B_2, explain the variations measured in nutrient intake.

Protein intake was the same among the animals given the raw pea diet (RP), those consuming the control diet of peas with no added phytase (PNP), and those given the phytase supplement (PP). The intake of soluble sugars was significantly lower among the animals given the diet of processed peas. On the other hand, the intake of available starch and total utilizable sugars was significantly higher among the animals given the processed diets (PNP and PP), there being no significant differences between these two groups. The intake of vitamins B_1 and B_2, α-galactosides, and trypsin inhibitors was significantly higher among the animals given the raw pea diet (RP) than among those consuming the PNP and PP diets, there being no significant differences between the latter two groups. The intake of phytic acid fell significantly as a consequence of the

Table 1. Effect of Phytase Addition on the Nutrient and Antinutritional Factor Contents of Peas[a]

	pea flour		
	raw (RP)	no phytase addition (PNP)	phytase addition (PP)
nitrogen (g/100 g of DM)			
total N	4.44 ± 0.05b	4.18 ± 0.04a	4.12 ± 0.05a
insoluble N	0.48 ± 0.01a	0.42 ± 0.02a	0.43 ± 0.01a
protein N	3.30 ± 0.02b	3.39 ± 0.03b	3.03 ± 0.03a
nonprotein N	0.66 ± 0.02b	0.37 ± 0.03a	0.57 ± 0.03b
available soluble sugars (g/100 g of DM)			
fructose	NDa	NDa	0.08 ± 0.01b
glucose	NDa	0.06 ± 0.01b	0.16 ± 0.01c
galactose	NDa	NDa	0.12 ± 0.01b
sucrose	1.73 ± 0.14c	0.32 ± 0.01a	0.38 ± 0.02b
total available soluble sugars	1.73 ± 0.14c	0.38 ± 0.01a	0.74 ± 0.03b
starch (g/100 g of DM)			
total starch	42.65 ± 0.58a	48.73 ± 0.91b	48.09 ± 0.10b
available starch	38.70 ± 1.21a	45.21 ± 0.60b	44.11 ± 0.66b
resistant starch	3.95 ± 0.65a	3.53 ± 0.30a	3.98 ± 0.57a
total available sugars	40.43 ± 0.36a	45.58 ± 0.31c	44.85 ± 0.35b
vitamins (mg/100 g of DM)			
B_1	0.729 ± 0.013b	0.217 ± 0.004a	0.216 ± 0.003a
B_2	0.146 ± 0.007b	0.088 ± 0.002a	0.090 ± 0.005a
α-galactosides (g/100 g of DM)			
raffinose	0.56 ± 0.03b	0.15 ± 0.01a	0.12 ± 0.01a
stachyose	2.24 ± 0.06b	0.45 ± 0.01a	0.43 ± 0.01a
verbascose	2.39 ± 0.10b	0.26 ± 0.01a	0.27 ± 0.01a
total α-galactosides	5.19 ± 0.13b	0.86 ± 0.01a	0.82 ± 0.02a
phytic acid (6-inositol phosphate) (g/100 g of DM)	0.339 ± 0.006c	0.075 ± 0.001b	0.025 ± 0.001a
trypsin inhibitor activity (TIU·mg^{-1} of DM)	8.69 ± 0.01c	2.45 ± 0.10b	2.19 ± 0.03a

[a] The same letter in the same row indicates no significant difference ($P < 0.05$). Values are means ± SD ($n = 3$). DM, dry matter.

Table 2. Nutrient and Antinutritional Factor Intake (Rat/Day)[a]

	pea flour		
	raw (RP)	no phytase addition (PNP)	phytase addition (PP)
intake (g of DM)	10.72 ± 0.20a	11.20 ± 0.29a	11.25 ± 0.41a
intake (g/100 g of rat body wt/day)	9.08 ± 0.24a	9.53 ± 0.27a	9.20 ± 0.27a
protein (g) (N × 6.25)	2.97 ± 0.06a	2.92 ± 0.08a	2.90 ± 0.11a
available soluble sugars (g)	0.19 ± 0.004c	0.04 ± 0.00a	0.08 ± 0.00b
total starch (g)	4.57 ± 0.09a	5.46 ± 0.15b	5.41 ± 0.17b
resistant starch (g)	0.42 ± 0.01ab	0.40 ± 0.01a	0.45 ± 0.01b
available starch (g)	4.15 ± 0.08a	5.06 ± 0.14b	4.96 ± 0.16b
total available sugars (g)	4.33 ± 0.09a	5.10 ± 0.14b	5.05 ± 0.20b
vitamin B_1 (mg)	0.078 ± 0.002b	0.024 ± 0.001a	0.024 ± 0.001a
vitamin B_2 (mg)	0.016 ± 0.000a	0.010 ± 0.000b	0.010 ± 0.000b
total α-galactosides (g)	0.56 ± 0.01b	0.01 ± 0.003a	0.09 ± 0.00a
phytic acid (6-inositol phosphate) (mg)	36.349 ± 0.72c	8.39 ± 0.23b	2.81 ± 0.09a
trypsin inhibitor (TIU)	93148 ± 1775b	27427 ± 717a	24637 ± 901a

[a] The same letter in the same row indicates no significant difference ($P < 0.05$). Values are means ± SEM of 10 Wistar rats. DM, dry matter.

mild hydrothermal treatment and was significantly lower within the group that consumed peas treated with phytase (PP) than in the other groups. The weight gain in grams/rat/day and the growth efficiency coefficient (PER) were significantly higher among the animals given the control diet with no added phytase (PNP) than among those that received the RP and PP diets, there being no significant differences between the latter two groups (**Table 3**). A similar pattern was found for the food transformation index (FTI), the values being lower among the animals of the control group, with no added phytase. The digestive and metabolic utilization of nitrogen is described in **Table 4**. The fecal excretion of nitrogen was significantly higher among the rats that were fed raw peas (RP) than with those given processed peas (PNP and PP). There were no significant differences between the two latter groups. The digestive utilization of nitrogen (ADC) was significantly higher in the PNP and PP groups than among those fed raw peas, with analogous results being obtained as a result of processing. The urinary excretion

Table 3. Weight Gain and Nutritive Utilization Coefficients of Protein of Rats Fed Raw and Processed Pea Diets[a]

	pea flour		
	raw (RP)	no phytase addition (PNP)	phytase addition (PP)
Δwt (g/rat/day)	1.90 ± 0.11a	3.22 ± 0.24b	1.99 ± 0.38a
PER	0.64 ± 0.03a	1.10 ± 0.06b	0.66 ± 0.11a
FTI	5.79 ± 0.28b	3.61 ± 0.20a	7.14 ± 1.20b

[a] The same letter in the same row indicates no significant difference ($P < 0.05$). Values are means ± SEM of 10 Wistar rats.

Table 4. Digestive and Metabolic Utilization of Nitrogen[a]

	pea flour		
	raw (RP)	no phytase addition (PNP)	phytase addition (PP)
daily N intake (mg/rat/day)	476 ± 9.07a	468 ± 12.23a	466 ± 17.04a
daily total fecal N (mg/rat/day)	78.2 ± 3.32b	61.3 ± 3.40a	63.1 ± 1.74a
total urinary N (mg/rat/day)	239 ± 9.50a	215 ± 9.52a	241 ± 8.27a
daily absorbed N (mg/rat/day)	398 ± 9.65a	406 ± 12.60a	403 ± 16.31a
ADC (%)	83.5 ± 0.78a	86.8 ± 0.78b	86.4 ± 0.44b
balance[b] (mg/rat/day)	159 ± 9.23a	191 ± 9.27b	161 ± 15.10a
% R/A[c]	39.9 ± 2.02a	46.93 ± 1.77b	39.6 ± 2.60 a

[a] The same letter in the same row indicates no significant difference ($P < 0.05$). Values are means ± SEM of 10 Wistar rats. [b] Balance = N intake − (fecal N + urinary N). [c] % R/A = [balance/(N intake − fecal N)] × 100.

Table 5. Composition of Liver and Longissimus Dorsi Muscle of Rats Fed Raw and Processed Pea Diets[a]

	pea flour		
	raw (RP)	no phytase addition (PNP)	phytase addition (PP)
liver			
% water	70.77 ± 0.19ab	71.16 ± 0.25b	70.45 ± 0.18a
N (% of DM)	11.67 ± 0.13ab	11.63 ± 0.15a	12.04 ± 0.08 b
longissimus dorsi muscle			
% water	73.31 ± 0.17a	73.24 ± 0.37a	74.10 ± 0.27a
N (% of DM)	14.69 ± 0.18a	14.49 ± 0.18a	14.71 ± 0.08a

[a] The same letter in the same row indicates no significant difference ($P < 0.05$). Values are means ± SEM of 10 Wistar rats. DM, dry matter.

of nitrogen was similar among all groups tested. The nitrogen balance was significantly higher in the control group, with no added phytase (PNP), than in the raw pea diet group (RP), with no significant differences being found between the RP and PP groups. The % R/A values were similar among the rats given the raw pea diet (RP) and those receiving the phytase addition (PP) and significantly greater in the control group, with no added phytase.

Table 5 describes the water and nitrogen contents in the liver and longissimus dorsi muscle of the rats given the raw and processed pea diets. In the liver of the animals given the control diet, with no added phytase (PNP), the water content was slightly higher ($P \leq 0.05$) than in the animals receiving the phytase supplement. Among the former animals, the nitrogen content in the liver was significantly lower. The water and nitrogen contents in the longissimus dorsi muscle were similar in all of the animals studied.

DISCUSSION

Chemical Analysis. With respect to the protein content, there was a nitrogen loss in the two diets of processed peas (PNP and PP) as a result of the mild hydrothermal treatment applied to the legume flour, which produced changes in the pH (from 6.3 in RP to 5.6 and 5.7 in PNP and PP, respectively).

Under the experimental conditions, the control diet, with no added phytase (PNP), had a lower non-protein nitrogen content soluble in the basic pH used for its extraction. This non-protein nitrogen is normally composed of free amino acids, peptides, or low molecular weight proteins, purine and pyrimidine bases, and alkaloids (*16*). The addition of phytase, however, in the raw pea diet group produced decreases in the basic pH-soluble protein nitrogen fraction without affecting the insoluble nitrogen fraction.

The treatment process increased levels of total and available starch, despite the decreased levels of available soluble sugars because it facilitated the dissociation and fragmentation of starch granules (*17*, *18*). These results could be due also to the low solubility of starch in mild hydrothermal conditions and the removal of the soluble available sugars with the discarded processing liquid; therefore, a rise in starch content, on a percentual basis, was achieved. Frias (*19*) reported an increment in total and available starch of lentils after 9 h of soaking at room temperature.

The high loss rate of vitamins B_1 and B_2 was due to solubilization and is analogous to that found by Frías et al. (*14*) in lentils. The addition of phytase did not produce any additional loss of the above vitamins.

The fall in the phytic acid content to 78% in the control diet, with no added phytase, was similar to that obtained for other legumes using processes such as soaking and cooking (*20*), germination (*21*), and fermentation (*15*). This reduction may be due to the fact that under the experimental conditions, the endogenous phytase present in the legume was activated (*22*, *23*) or may be a consequence of the mild hydrothermal treatment itself. The addition of phytase to the diet reduced the phytic acid content even more (93% with respect to the RP diet), as the preparation of this diet was carried out under optimal experimental conditions intended to achieve a greater hydrolysis of phytic acid, following previous studies (*24*).

Many studies have detected the almost complete loss of trypsin inhibitors caused by thermal processes such as extrusion [peas, from 6.32 to 0.34 TIU·mg^{-1} of DM (*25*)] and soaking and cooking [faba beans, from 2.62 to 0 TIU·mg^{-1} of DM (*26*)]. Under the present experimental conditions, the reduction in trypsin inhibitors was 70%, which we believe is due to the solubilization caused by the treatment, as the TIA is not affected by the 37° C temperature applied (*27*).

In an analogous way, the existence of lower α-galactoside levels in the processed diets is mainly due to the methodology of the treatment itself and not exclusively to the action of phytase. As a result of the mild hydrothermal treatment, metabolic changes lead to a reduction in α-galactoside levels (*28*). Moreover, the reduction in the presence of these compounds is greater when mild hydrothermal treatment is applied because the supernatant is discarded, these carbohydrates being solubilized in the treatment liquid. Our results coincide with those of Iyer et al. (*29*), who found that soaking lowered the concentration of α-galactosides, trypsin inhibitors, and phytic acid in different varieties of *Phaseolus vulgaris*.

Biological Analysis. *Intake.* Mild hydrothermal treatment and the addition of phytase to the pea diets produced a significant fall in the levels of α-galactosides, but no increase in food intake. In other legumes, such as faba beans (*30*) and chickpeas (*31*) subjected to soaking in water and in a basic medium, there was found to be an increase in food intake, due to the lower levels of α-galactosides. In the PNP diet (pH 5.6) and the PP diet (pH 5.7), there was a decrease in the pH, which might have masked the increased food intake expected from the processed diets with lower levels of α-galactosides (*32*).

Digestive Utilization of Protein. The rate of digestive utilization of protein in peas is high, similar to that found for faba beans (*30*) and much higher than that of lentils, chickpeas, and beans (*32*−*34*). The improvement in the digestive utilization of protein (83.5−86.8%) by the application of mild hydrothermal treatment is attributed to the diminution in TIA (70%) and in phytic acid (78%). Phytic acid forms complexes with proteins at both acidic and basic pH values (*1*). The nutritional implications of these phytate−protein complexes are related to the lower solubility of the protein that is produced, which could adversely affect certain functional properties of legumes that depend on their hydration and solubility and which make them more resistant to proteolytic degradation (*35*, *36*). In the present experimental conditions, the addition of phytase produced an additional diminution of phytic acid by 16% to reach a total reduction of 93%, but this did not lead to a subsequent improvement in the digestive utilization of protein.

Metabolic Utilization of Protein. The treatment applied significantly improved the balance and the % R/A in the group of rats receiving the PNP diet. The addition of phytase produced no visible improvement of these indices with respect to the group of animals given the raw pea diet and may interfere with the

nutritive utilization of protein, with an adverse effect on its retention and metabolic utilization. These results should be considered taking into account the weight gain data and the PER and FTI coefficients (**Table 3**), which show that the rats given the PNP diet grew better than those receiving the other two experimental diets in the sense of weight gain associated with protein gain; as is well-known, an increase in weight may be due to a greater amount of fat or to water retention, two factors that are not related to growth as such. The greater weight gain of the animals consuming the PNP diet cannot be attributed to a greater food intake, as this was similar for all three groups (**Table 2**), nor to a higher consumption of fats because this macronutrient, a fundamental source of energy, was added in equal quantities to all of the experimental diets. The greater degree of growth observed was probably the result of a better nutritive utilization of carbohydrates, which enabled the animals to retain dietary protein to be used for growth and to use fats and carbohydrates as the main energy source.

Chemical analysis of the diets reveals that, in the PNP and PP diets, the treatment considerably increased the quantities of total and available starch, whereas resistant starch was not modified. When phytase was added to the PNP diet, in some way it prevented the total available sugars from being utilized with the same metabolic efficiency. In conclusion, with the PP diet, the animals consumed the same volume of available carbohydrates but made a less efficient use of these macronutrients, which had a substantial effect on the final growth achieved.

Effects on Various Organs. On studying the composition of the liver, we found that the water content was significantly higher in the control group (no added phytase). It is well-known that 4 g of water is stored in the liver for every gram of glycogen (*37*). This greater amount of water could indicate that more carbohydrates are stored in the livers of these experimental rats, which would agree with the hypothesis that the control treatment, with no added phytase, facilitates the nutritive utilization of carbohydrates and leads to greater weight gain.

Although the addition of phytase does not produce significant improvements in the indices of nutritive utilization of protein, it does increase the amount of nitrogen stored in the liver.

With respect to the analysis of the longissimus dorsi muscle, no significant changes in the content of nitrogen or water were observed.

In conclusion, the 70% reduction in the levels of phytic acid, produced by the mild hydrothermal treatment applied, improved the digestive utilization of protein, but no additional improvement occurred when the levels of phytic acid decreased by 93% after phytase was added. Mild hydrothermal treatment increased the nutritive utilization of protein and carbohydrates, but this effect was not observed in the phytase-supplemented diet.

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