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Effect of Composition and Processing on the Nutritive Value of Some Leguminous Seeds

R. PION, E. MENOLES-PEREIRA, and **J. PRUGNAUD,** I.N.R.A. – *Laboratoire d' Etude du Métabolisme Azoté, THEIX 63110, Beaumont, France*

ABSTRACT

Leguminous seeds are potential protein sources with variable nutritive value because of their amino acid pattern. Their nutritive value may be affected by some nonprotein components: tannins, poorly digestible carbohydrates and antinutritional factors. Some deleterious effects may be avoided by different technological processes developed to eliminate or inactivate the indesirable compounds. Unfortunately, the processes may themselves result in a decrease of the nutritive value of the proteins by making their amino acids unavailable to the animal organism. We studied some of these effects in field beans, soybeans, lupines and peas, using digestibility and growth trials with rats. The samples were used as sole proteins in balanced diets and supplemented with synthetic amino acids to meet the requirements. The significance of differences in apparent digestibilities was estimated from the amino acid composition on the feces using two calculation procedures described previously. These techniques were developed to assess the relative part of the undigestible food and of the intestinal bacteria in the feces. The growth of the animals was compared to a control group eating a fish meal diet. The free amino acids in blood and muscle were estimated in order to emphasize some amino acid unavailability.

INTRODUCTION

We previously reported some experiments with rats related to the nutritive value of some raw and processed seeds, as well as protein concentrates and isolates, and textured proteins (1-3). We summarize in the present paper results obtained with leguminous seeds, with and without processing. We use as the main criteria interpretation of the amino acid composition of the feces and the blood and muscle free amino acid contents (4,5).

MATERIALS AND METHODS

The samples under study were soybean commercial products (isolate, concentrate, textured protein) differing by their nitrogen contents, whole horse beans, peas and lupines either raw or cooked, hulled horse beans with or without heat treatment, extruded high protein horse bean flour.

The animals were male weanling S.P.F. rats of the Sprague-Dawley strain, 21 days old, housed in individual cages. The temperature (22 C) and relative humidity (60%) were controlled. They received during a six day period a commercial standard diet, and were divided into experimental groups of 5 rats in the balance trials and of 10 rats in the growth experiments. In all the experiments, the animals were fed the experimental diets during seven days before the beginning of the experiments.

The samples were used as sole protein source in semiliquid diets supplemented with synthetic amino acids in order to meet the amino acid requirements. The diets were approximately isonitrogenous and made isoenergetic with wheat starch and peanut oil. A standard vitamin and trace mineral mixture (6) was added. The minerals (Na, K, P, Mg, Ca) lacking in the samples were added. In the digestibility experiments, the feces were collected during five days. In the growth experiments, the compositions of the diets were corrected for nitrogen digestibility. The diets made from high protein foodstuffs had a high wheat starch content, and the others contained important amounts of the samples own carbohydrates. Food intake was measured daily during the 10 day experimental period, and body weights were meausred three times each week. At the end of the experiments, the animals were killed between 9 and 10 a.m.

TABLE I

Digestion of Some Soybean Samples

Sample	Textured	Concentrate	Isolate
Sample crude protein % dry matter	55.7	69.7	91.7
Dietary crude protein % dry matter	14.25	14.0	12.85
Apparent digestibility			
– sample nitrogen	84	79.9	90.4
– diet organic matter	92.8	90.2	94.5
ARD			
– feces/bacteria	13.9	14.4	15.0
– feces/food	25.2	26.5	24.8
Regression coefficients			
a (food)	0.15[a]	0.15	0.29[a]
b (bacteria)	1.0	1.0[a]	0.80[a]
K	-0.81	-0.82	-0.46
R^2	0.96[a]	0.95[a]	0.95[a]

[a]$P < 0.01$.

TABLE II

Digestion of Some Horse Bean Samples

Samples	S 45 Raw	S 45 Steam-cooked	Ascott hulled Raw	Ascott hulled Micronized	Ascott hulled Steam-flaked	High protein flour-extruded
Sample crude protein % dry matter	27.4		37.2	36.7	35.3	55.9
Dietary crude protein % dry matter	15.6	15.7	13.6	14.05	14.15	14.9
Apparent digestibility						
– sample nitrogen	70.9	77.0	86.0	84.9	86.9	89.0
– diet organic matter	80.8	83.8	94.8	94.6	95.1	93.2
ARD						
– feces/bacteria	19.6	11.1	10.1	11.1	11.3	8.5
– feces/food	29.1	25.8	31.05	30.8	30.8	41.0
Regression coefficients						
a (food)	0.34^a	0.21^a	0.15^b	0.16	0.16	0.07
b (bacteria)	0.52^a	0.75^b	1.06^b	0.95^b	0.87^b	0.99^b
K	0.80	0.18	-1.22	0.62	0.14	0.38
R^2	0.81^b	0.94^b	0.92^b	0.93^b	0.92^b	0.94^b

$^a P < 0.05$.
$^b P < 0.01$.

TABLE III

Digestion of Some Pea and Lupine Samples

Sample	Peas Frogel Raw	Peas B2 Steam-cooked	Lupine Yellow (Weiko) Raw	Lupine Yellow (Weiko) Steam-cooked	Lupine White Lucky	Lupine White Kali	Lupine White Kalina	Lupine Blanca Raw	Lupine Blanca Steam-cooked
Sample crude protein % dry matter	26.5	29.1	42.7	42.7	34.6	39.2	39.2	36.9	36.9
Dietary crude protein % dry matter	13.0	13.7	14.1	15.3	13.4	13.5	13.6	15.1	16.0
Apparent digestibility									
– sample nitrogen	78.4	78.9	84.7	84.5	78.5	82.0	80.8	80.4	78.3
– diet dry matter	84.35	87.2	89.2	88.3	84.7	87.1	85.9	82.2	82.0
ARD									
– feces/bacteria	9.1	8.3	10.9	12.4	13.3	11.2	11.3	11.6	10.4
– feces/food	25.7	29.2	35.9	40.4	34.3	36.4	33.3	36.0	37.1
Regression coefficients									
a (food)	0.11	0.11	0.20^a	0.12	0.16	0.19^a	0.17^b	0.14	0.08
b (bacteria)	1.01^a	0.91^a	0.89^a	0.92^a	1.04^a	0.99^a	0.95^a	0.95^a	0.95^a
K	-0.63	-0.13	-0.62	0.25	-1.12	-0.94	-0.9	-0.59	-0.27
R^2	0.96^a	0.95^a	0.96^a	0.93^a	0.93^a	0.96^a	0.95^a	0.92^a	0.93^a

$^a P < 0.01$.
$^b P < 0.05$.

and samples of blood and muscle of the hind limbs were taken. Whole samples were immediately mixed with cold ethanol, and muscles were frozen in liquid nitrogen. All samples were stored at -15 C.

Nitrogen was determined by the Kjeldahl method; amino acids from the food and from the feces were determined after hydrolysis with HCl (500 ml of HCl 5.5 N for 400 mg of dry matter) by ion exchange column chromatography. Performic acid oxidation before hydrolysis was used for sulfur amino acids. Free amino acids were extracted from blood with 84% ethanol, and from muscle with 1% picric acid as described previously (6), and estimated by ion exchange column chromatography with an automatic apparatus.

RESULTS AND DISCUSSION

Digestibility Experiments

The apparent digestibilities of nitrogen and dry matter were calculated (Tables I, II, III). In order to appreciate if the differences between the apparent nitrogen digestibility values of the diets are related to dietary protein excretion or to intestinal bacteria and endogenous protein excretion, we used two calculation procedures (7,8).

We calculated the average relative differences (A R D) between fecal amino acid compositions and dietary protein or intestinal bacteria amino acid compositions. (9) The A R D between two proteins i and j is calculated as follows:

$$ARD = \frac{1}{n} \sum_{K=1}^{n} \frac{\sqrt{(AA_{ik} - AA_{jk})^2}}{\frac{AA_{ik} + AA_{jk}}{2}} \times 100$$

where n is the number of amino acids taken into account, and AAik and AAjk are the respective percentages of the amino acid k in the proteins i and j. Tryptophan and cystine values were omitted from the calculations: tryptophan was not estimated in the feces samples, and the cystine values may be overestimated because of undigested hairs in the feces.

The influence of dietary proteins on the amino acid composition of the feces was also estimated by calculating a multiple regression ($y = ax1 + bs2 + c$) where Y, x1 and x2 are the respective amino acid contents of fecal protein, dietary protein, and microbial protein. They take the values

TABLE IV

Average Weight Gain of Rats Given Some Legume Seed Diets

Sample	Horse beans						Peas	Lupines	
	Whole beans			High protein-extruded meals					
	Bianka		S45				B2	White (blanca)	
Treatment	Raw	Cooked	Cooked				Cooked	Raw	Cooked
Dry matter intake g/day	16.71 ± 1.99	17.61 ± 1.81	13.53 ± 2.27	17.45 ± 1.85	16.98 ± 2.05	14.65 ± 3.23	16.98 ± 1.99	16.52 ± 1.65	13.40 ± 2.04
Weight gain g/day	5.83 ± 0.83	5.53 ± 1.03	3.92 ± 0.99	5.65 ± 1.31	6.44 ± 1.02	4.90 ± 1.44	5.89 ± 0.91	5.07 ± 0.93	4.27 ± 0.87
Weight gain % control	98.9	93.9	61.1	95.9	104.4	79.4	100.0	78.9	66.5

estimated in these proteins for each amino acid.

Results obtained with some commercial soybean samples may be seen in Table I: the nitrogen digestibility is better for the isolate (more than 90% crude protein) than for the concentrate or the textured protein. In all cases, the ARD values between fecal and ingested proteins are higher than those between fecal and bacterial proteins: thus, the amino acid compositions of the feces are closer to that of intestinal bacteria than to that of ingested proteins. It may be inferred from the regression coefficients that all feces samples contain significant amounts of bacterial proteins; on the other hand, a significant amount of dietary amino acids seems to occur only as far as the protein isolate is concerned.

The apparent nitrogen and organic matter digestibilities of whole S 45 high tannin horse beans (Table II) are very low; they are greatly increased by cooking. It may be seen from the ARD values that the amino acid compositions of the feces are closer to that of intestinal bacteria for the rats given the cooked beans than for those given the raw ones. Moreover, as the amino acid content of the feces strongly differs from that of the dietary proteins as far as the raw beans are concerned, most of the excreted amino acids may be of endogenous origin. However, it may be inferred from the regression coefficients that the fecal proteins contain significant amounts of bacterial amino acids as well as dietary amino acids. The cooking of the beans decreases dietary amino acid excretion and increases the proportion of bacterial proteins in the feces.

The apparent digestibilities of hulled beans are high and not significantly modified by heat treatment. It may be seen from the ARD values as well as from the regression coefficients that the amino acid compositions of the feces of the rats given these beans look like that of bacteria and strongly differ from that of ingested proteins.

The apparent digestibilities of pea and lupine samples (Table III) are in most cases better than that of cooked whole horse beans. They are not improved by cooking. The amino acid compositions of the feces are close to that of bacterial proteins, as may be inferred from the ARD values as well as from the regression coefficients. However, significant amounts of dietary amino acids seem to appear with the three lupine samples.

Thus, the calculation procedures we used make us able to know if differences in apparent nitrogen digestibilities are mainly related to differences in dietary amino acid or in bacterial amino acid excretion. However, we need more accurate estimations of the amino acid patterns of bacterial and especially endogenous proteins. It is also necessary to know how these patterns may be modified by dietary proteins as well as by dietary carbohydrates.

Growth Experiments

Due to the variability from one trial to another in gains of body weight by rats given the same diet, the results were compared to that obtained in the same experiment with a starch-fish meal control diet (Table IV).

The high tannin S 45 cooked horse beans were compared to tannin less horse beans Bianka, either raw or cooked: the occurrence of tannins in the seeds seems to strongly decrease the growth of the animals. The daily weight gains obtained with two of the high protein horse bean-extruded meals and the pea B2 were good. On the contrary, the growth rates of the rats given the white lupine diets were low and decreased with cooking. Thus, though the diets were supplemented with industrial amino acids to meet the needs, some of them were unable to allow maximum growth of the animals.

The free amino acid contents of blood and muscle (Table V) did not explain the low growth rate of the rats given the S 45 horse bean diet; it is probably mainly related to bad digestion processes. On the other hand, the low

TABLE V

Free Amino Acids in Blood and Muscle of Rats Given Horse Bean Diets

Sample	Whole bean					
	Bianka		S45			
Treatment	Raw	Cooked	Cooked	High protein-extruded meals		
Blood-free amino acids mg/100 g whole blood						
Threonine	3.65	3.19	2.42	1.58	1.99	1.11
Valine	1.67	1.48	1.24	1.36	1.67	1.32
Isoleucine	0.99	0.94	0.74	0.91	0.94	0.73
Leucine	1.31	1.30	1.20	1.25	1.50	1.14
Tyrosine	1.56	1.57	1.19	1.58	1.41	1.27
Phenylalanine	0.85	0.85	0.81	0.90	0.92	0.71
Lysine	4.54	4.95	5.64	5.26	5.70	6.54
Histidine	1.08	1.14	1.23	1.16	1.20	1.15
Arginine	3.50	4.01	4.75	3.26	4.16	4.66
Muscle-free amino acids mg/100 g						
Threonine	12.69	12.92	11.43	6.55	6.5	4.45
Valine	2.57	2.24	1.73	2.43	1.60	1.50
Isoleucine	0.92	1.02	1.20	1.06	1.11	0.98
Leucine	1.39	1.01	1.69	1.35	1.49	1.31
Tryosine	2.12	1.58	2.03	2.60	2.03	2.18
Phenylalanine	1.10	0.79	0.92	1.15	0.87	0.83
Lysine	17.44	17.50	25.99	14.4	13.6	20.9
Histidine	5.02	5.81	6.23	4.35	4.13	4.72
Arginine	12.90	14.42	17.06	7.2	10.2	9.7

TABLE VI

Free Amino Acids in Blood and Muscle of Rats Given Pea and Lupine Diets

Sample	Peas B2	White lupines	
Treatment	Cooked	Raw	Cooked
Blood-free amino acids mg/100 g whole blood			
Threonine	2.96	5.33	6.16
Valine	1.46	1.28	1.60
Isoleucine	0.86	0.91	1.01
Leucine	1.14	1.21	1.81
Tyrosine	1.17	1.19	2.03
Phenylalanine	0.78	0.80	0.99
Lysine	5.34	5.35	5.49
Histidine	1.01	1.00	0.95
Arginine	4.40	4.44	5.30
Muscle-free amino acids mg/100 g			
Threonine	10.43	24.18	23.75
Valine	2.14	1.69	1.74
Isoleucine	0.90	1.23	1.33
Leucine	0.77	1.64	1.71
Tyrosine	1.47	1.72	2.79
Phenylalanine	0.81	0.92	0.98
Lysine	28.36	26.58	26.35
Histidine	5.14	4.20	4.68
Arginine	22.20	19.85	24.92

value observed for the third high protein horse bean-extruded meal may be due in part to the unavailability of part of the dietary threonine, as it may be inferred from the values of blood and muscle threonine. Moreover, the low blood and muscle threonine contents observed for the two other high protein meals emphasize unavailabilities that were not strong enough to decrease the growth rates of the animals. On the other hand, the low values of the growth of the rats given the lupine diets seem not to be related to the unavailability of one of the tabulated amino acids.

Thus, the estimation of blood- and muscle-free amino acids may point out some unavailabilities of amino acids; it also made us able to see that the technological processes may impair the availabilities of amino acids other than lysine, like threonine.

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