FEEDING STUDIES ON SOYBEANS. GROWTH AND PANCREATIC HYPERTROPHY IN RATS FED SOYBEAN MEAL FRACTIONS[1]

J. J. RACKIS, A. K. SMITH, A. M. NASH, D. J. ROBBINS,[2] AND A. N. BOOTH[2]

ABSTRACT

Rats were fed raw or toasted soybean meal, acid-precipitated protein, whey solids, and whey protein. Weight gains, protein efficiencies, and pancreas weights were determined. As measured chemically, all raw products contained widely different levels of trypsin inhibitor activity; and for some fractions there was no direct relationship between trypsin inhibitor activity, growth inhibition, and pancreatic hypertrophy. Autoclaving of the meal and residue significantly increased weight gains and protein efficiency values, and decreased pancreatic hypertrophy. The residue contains a pancreatic hypertrophic factor that is relatively heat-stable. Autoclaving had no effect on the nutritive value of the acid-precipitated protein. Only slight pancreatic hypertrophy occurred in rats fed raw acid-precipitated protein.

Poor growth, low protein efficiency, and pancreatic hypertrophy occurred with rats fed casein diets containing raw soybean whey solids and whey protein. Trypsin inhibitor activity of these diets was very high. Better weight gains and higher protein efficiency values were obtained with casein supplemented with toasted whey protein than with casein alone. Significant pancreatic hypertrophy also occurred in rats fed autoclaved whey protein.

A number of heat-labile substances, physiologically active, have been reported in soybean meal and reviewed by Liener (6). In more recent experiments, Booth *et al.* (1) reported that when raw meal was fed to rats as a sole source of dietary protein, the adverse effects noted were (a) poor growth, (b) low food efficiency, and (c) pancreatic hypertrophy.

Factors in raw soybean meal that are responsible for poor growth and pancreatic hypertrophy are readily inactivated by autoclaving the meal with live steam for as little as 15 min. at atmospheric pressure.

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[2]Western Regional Research Laboratory, Albany, California, a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

In an attempt to isolate and determine these factors, raw meal was fractionated into residue, acid-precipitated protein, whey solids, and whey proteins. Because soybeans and soybean protein products are becoming an increasingly important source of human food (11), further information on the nutritional value of these fractions was desired from the feeding studies made with rats. The study included not only the relationship between growth, pancreatic hypertrophy, and trypsin inhibitor activity, but also the effect of autoclaving on the nutritive value of the residue, acid-precipitated protein, whey solids, and whey protein. For comparative purposes commercial acid-precipitated proteins were also evaluated.

Experimental

Preparation of Soybean Meal Fractions. A schematic diagram of the preparation of meal fractions from Hawkeye soybeans, 1959 crop, is shown in Fig. 1. Yields, nitrogen, and protein contents of the residue,

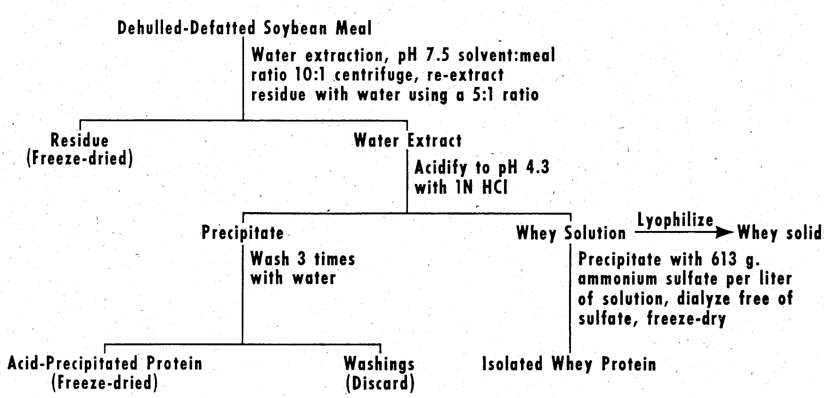

Fig. 1. Preparation of soybean meal fractions.

acid-precipitated protein, whey solids, and whey protein used in this study are given in Table I.

Commercial acid-precipitated proteins A, B, and C, commonly called isolated soybean protein, were obtained from three processors. The general methods for their preparation are similar to the laboratory procedure for the isolation of acid-precipitated protein D, as outlined in Fig. 1. The differences are probably in pH and water-to-meal ratio used in extraction, as well as the amount of washing of the wet curd and the amount of heat-treatment. These proteins are food-grade. Vegetable protein isolates have been described in a review by Smith (10).

TABLE I

YIELD, NITROGEN, AND PROTEIN CONTENTS OF SOYBEAN RESIDUE, ACID-PRECIPITATED PROTEIN, WHEY SOLIDS, AND WHEY PROTEIN FRACTIONS

FRACTION	YIELD[a]	NITROGEN[a]	PROTEIN (N × 6.25)
	g./100 g. meal	%	%
Meal	. . .	9.23	57.8
Residue	31.0	7.14	44.6
Acid-precipitated protein:			
A	[b]	14.75	92.2
B	[b]	16.09	100.1
C	[b]	14.25	89.1
D	35.9	15.93	99.6
Whey solids	33.1	3.86	12.82[c]
Whey protein	3.0	15.70	98.1

[a] Dry basis.
[b] Not known, however, yields obtained commercially are lower than D.
[c] Corrected for nonprotein nitrogen.

"Raw" refers to those fractions that have not received any heat-treatment during their preparation, whereas "toasted" designates that the fractions were heated in an autoclave with steam for 30 min. at atmospheric pressure.

Methods

Weanling male albino rats, separated into groups of five weighing 40 to 50 g., were housed in individual wire-bottomed cages and fed *ad libitum*. The basal diet composition is given in Table II.

TABLE II

COMPOSITION OF BASAL DIET

INGREDIENT	PERCENT OF DIET
Crude casein[a]	17.2 (N × 6.25 = 14% protein)
Cerelose	50.8
Corn starch	20.0
Soybean oil	4.0
Salt, USP XIV	4.0
Vitamin mix[b]	2.0
Powdered cellulose	2.0

[a] Soybean ingredients were substituted for casein, cerelose, and powdered cellulose in amounts necessary to maintain the same protein content as the basal diet.
[b] Vitamin mixture supplied the following nutrients per 100 g. of diet: 1,800 units vitamin A, 200 units vitamin D, 10 mg. alpha-tocopherol, 90 mg. ascorbic acid, 10 mg. inositol, 150 mg. chlorine chloride, 4.5 mg. menadione, 10 mg. *p*-aminobenzoic acid, 9 mg. niacin, 2 mg. riboflavin, 2 mg. pyridoxine hydrochloride, 2 mg. thiamine hydrochloride, 6 mg. calcium pantothenate, 0.04 mg. biotin, 0.18 mg. folic acid, and 0.0027 mg. vitamin B-12.

Weekly records of body weight and food intake for each rat were maintained. Protein efficiency (PE) was calculated by dividing the gain in weight by the protein intake. A 35-day feeding assay was employed. Rats were then sacrificed and pancreatic tissue, including extraneous fat, was excised and preserved in 10% aqueous formalin for 48 hr., after which the tissue was carefully trimmed, blotted, and weighed. All feeding experiments described were repeated at least once. Fresh preparations of the soybean products were used in the repeat experiments. No significant variations in the data were obtained between experiments. All statistical evaluations of the experimental soybean diets were made with respect to the casein control diet. Trypsin inhibitor activity was determined according to Kunitz (5) and is reported as units equivalent to the milligrams of 5X crystallized soybean trypsin inhibitor required to inhibit the same amount of trypsin. The inhibitor purchased from Nutritional Biochemical Corporation,[3] Cleveland, Ohio, was of at least 95% purity, according to the chromatographic procedure of Rackis *et al.* (9).

Results

Effects of Soybean Residue and Laboratory-Prepared and Commercial Acid-Precipitated Proteins. Weight gains and PE of raw and toasted residue and of acid-precipitated proteins are given in Table III.

TABLE III

EFFECT OF SOYBEAN RESIDUE AND ACID-PRECIPITATED PROTEINS CONTAINING TRYPSIN INHIBITOR ON GROWTH, PROTEIN EFFICIENCY, AND PANCREAS WEIGHT OF RATS

Diet No.	Dietary Constituent	Mean Weight Gain ± SE [a]	PE [b]	Mean Pancreas Weight ± SE [a]	Trypsin Inhibitor Content
		g.		*g. /100 g. B.W.*	*mg./100 g. diet*
1	14% Casein [c]	94.4 ± 2.94	2.29	0.43 ± 0.05	...
2	Raw residue [d]	77.0 ± 7.64	1.53**	0.60 ± 0.03*	66
3	Toasted residue [d]	101.2 ± 4.52	2.04*	0.55 ± 0.006*	15
4	Toasted acid-pptd. protein D	65.0 ± 3.69**	1.63**	0.45 ± 0.02	12
5	Raw soybean meal	25.6 ± 5.74**	0.78**	0.78 ± 0.06**	629
6	14% Casein	114.6 ± 3.59	2.25	0.47 ± 0.02	...
7	Toasted soybean meal	122.2 ± 4.71	2.01	0.41 ± 0.02	29
8	Acid-pptd. protein A	94.2 ± 5.25*	1.82**	0.51 ± 0.03	63
9	Acid-pptd. protein B	91.6 ± 4.69**	1.65**	0.52 ± 0.02	18
10	Acid-pptd. protein C	88.2 ± 2.16**	1.76**	0.51 ± 0.03	13
11	Raw acid-pptd. protein D	69.4 ± 2.99**	1.40**	0.56 ± 0.04	260

[a] SE = standard error. B.W. = body weight.
[b] Protein efficiency was calculated by dividing the gain in weight by the protein intake.
[c] 35-day assay, mean initial weight of rats for diets 1 through 5 = 40.0; for diets 6 through 11 = 49.9.
[d] One half of the 14% protein level in diets 2 and 3 was casein; in all other diets soybeans were the sole source of protein.
$*P < 0.05$; $**P < 0.01$.

Replacement of 50% of the casein with raw residue decreased rat growth by 18% (diet No. 2) and significantly decreased PE ($P<0.01$) compared with the casein control diet (diet No. 1). Since toasting had a large beneficial effect on weight gain and PE of the residue, this effect

[3] Mention in this article of commercial companies or materials does not constitute endorsement by the U.S. Department of Agriculture.

indicated the residue contains a growth-inhibiting factor that is inactivated by heat (diet No. 3). There is significant pancreatic hypertrophy in rats fed both raw and toasted residue ($P<0.05$). This observation suggests that the pancreas-stimulating factor in the residue is relatively more heat-stable than the factor in raw meal, because no pancreatic hypertrophy occurs in rats fed toasted soybean meal (diet No. 7).

When toasted acid-precipitated proteins (diets 4, 8, 9, and 10) were used as the sole source of protein in the diet, weight gains and PE ($P<0.01$) were highly significantly reduced as compared with casein. Acid-precipitated protein has a PE value lower than that of toasted soybean meal (diet No. 7) if fed at the same protein level. Comparison of diets 4 and 11 shows that toasting has a comparatively small effect on PE. Only a slight enlargement of pancreas occurred in rats fed raw acid-precipitated protein in spite of appreciable amounts of trypsin inhibitor activity in this diet (No. 11), as measured chemically. Lower weight gains and PE were recorded with diets containing acid-precipitated protein, prepared in the laboratory, as compared with commercial soybean proteins. Commercial acid-precipitated proteins are prepared from several varieties of soybeans grown at various locations. Krober (4) has reported that soybeans differ significantly in methionine content, according to variety and location. A difference in methionine content is most likely responsible for the lower nutritive value of the laboratory-isolated protein.

In fact, the low PE of both toasted residue and acid-precipitated protein, when compared with casein, is most likely due to a greater methionine deficiency. Using the system followed by the Food and Agriculture Organization of the United Nations (2), Rackis and coworkers (8) reported on the basis of their amino acid data that the first limiting amino acids in the residue and acid-precipitated protein are cystine plus methionine. Both fractions have a protein score of 54 compared to 80 for casein.

Some feeding experiments were conducted with toasted, acid-precipitated protein diets, containing 0.3% added methionine. Protein efficiency values of 2.07 were obtained, and weight gains on these diets were slightly higher than those from casein (11). These results are consistent with the amino-acid compositional data of the residue and acid-precipitated proteins (8).

Effects of Whey Solids and Isolated Whey Proteins. Growth response data of rats fed both raw and toasted whey solids and isolated whey protein fractions are given in Table IV. In diets 13 and 14, whey solids

TABLE IV

EFFECT OF WHEY SOLIDS AND ISOLATED WHEY PROTEINS CONTAINING TRYPSIN INHIBITOR ON GROWTH, PROTEIN EFFICIENCY, AND PANCREAS WEIGHT OF RATS

Diet No.	Dietary Constituent	Mean Weight Gain ± SE[a]	PE[b]	Mean Pancreas Weight ± SE[a]	Trypsin Inhibitor Content
		g.		*g./100 g. B.W.*	*mg./100 g. diet*
12	12% Casein[c]	162.2 ± 6.06	1.96	0.43 ± 0.03	...
13	Raw whey solids (lyophilized)	108.4 ± 2.77**	1.36**	0.67 ± 0.04**	715
14	Raw whey solids (spray-dried)	118.3 ± 8.47**	1.45**	0.79 ± 0.05**	936
15	12% Casein[d]	173.0 ± 2.96	2.21	[e]	...
16	Raw whey solids (lyophilized)	148.6 ± 10.72	1.92**	[e]	385
17	Toasted whey solids (lyophilized)	157.6 ± 8.10	2.06*	[e]	25
18	14% Casein[f]	124.6 ± 10.20	2.15	0.52 ± 0.04	...
19	Raw whey protein (lyophilized)	69.8 ± 3.92**	1.53**	1.11 ± 0.09**	1,040
20	Toasted whey protein (lyophilized)	137.3 ± 3.57	2.28	0.69 ± 0.04*	<5

[a] SE = standard error. B.W. = body weight.
[b] Protein efficiency was calculated by dividing the gain in weight by the protein intake.
[c] Diets 12 through 14, a 51-day assay, whey solids account for 13% of the diet.
[d] Diets 15 through 17, a 54-day assay, whey solids account for 7% of the diet.
[e] Not determined.
[f] Diets 18 through 20, a 35-day assay, whey proteins account for 5% of the diet.
*P <0.05; **P <0.01.

accounted for 13% of the diet and replaced 20% of the casein. Whey solids at the 13% level correspond to the amount present in a raw soybean meal diet containing 14% protein as determined by actual yield of whey solids obtained in the laboratory. The results show that raw whey solids depress growth, reduce PE, and enlarge the pancreas. The amount of growth inhibition and pancreatic hypertrophy obtained in this experiment is comparable to that of a raw-meal diet containing the same amount of whey solids (unpublished data).

On the other hand, by reducing the raw whey solids in a casein diet from 13 to 7%, diets 13 and 16, respectively, significant decreases in weight gains and PE still occurred but not to the same extent. Only slight improvement was obtained with a toasted whey solids diet at the 7% level of incorporation. In these experiments whey solids were fed at the 7% level because it represents an actual yield of whey solids obtained in an industrial plant preparing acid-precipitated protein.

Raw whey proteins, which accounted for 5% of the diet and which replaced 32% of the casein in diet 19, resulted in poor rat growth, reduced PE, and pancreatic hypertrophy. Compared with the casein control diet, toasted whey proteins resulted in increased rat growth and PE was significantly higher. After autoclaving, however, the pancreas-stimulating factor still exerted a significant effect even though trypsin inhibitor content was very low (diet No. 20).

The high PE value for diets containing toasted whey proteins are attributed to a good balance of essential amino acids, particularly tryptophan, lysine, and cystine plus methionine, and to a protein score of 87 (8). During commercial processing of meal into acid-precipitated protein, whey proteins are a waste product, which must be treated as sewage.

Discussion

Haines and Lyman (3) reported that rats fed heated soybean meal supplemented with soybean trypsin inhibitor (activity equivalent to raw meal) showed depressed growth, but with less severity than when raw meal was fed. Both diets, however, produced the same accelerated pancreatic secretory response. Presumably, this increased secretion of pancreatic juice enzymes causes pancreatic hypertrophy. Active trypsin inhibitor preparations from egg white, lima beans, and soybeans stimulate the secretion of large amounts of pancreatic enzymes in the rat (7).

Our feeding tests indicate, however, that there may not be a direct correlation between the trypsin inhibitor activity and growth inhibition or pancreatic hypertrophy. For example, raw soybean meal diets containing high levels of trypsin inhibitor activity (Table III) depressed growth, reduced PE, and resulted in pancreatic hypertrophy. After the meal was toasted and with the inhibitor activity reduced considerably, the deleterious effects of raw meal were eliminated. On the other hand, the residue diets containing low levels of trypsin inhibitor activity, although nearly comparable to toasted soybean meal, can still cause pancreatic hypertrophy (see diets 2, 3, and 7). The acid-precipitated protein (diet 11) containing relatively large amounts of trypsin inhibitor activity had very little effect on growth, PE, and pancreas. Raw whey solids and whey proteins which also contain high levels of trypsin inhibitor activity will markedly reduce growth and PE and enlarge the pancreas. Toasted whey proteins still contain an active pancreatic hypertrophic factor.

These results suggest that the effect of soybean trypsin inhibitors on growth and the pancreas may depend upon the presence of other meal constituents. This, together with the fact that the pancreatic factor in the water-insoluble residue and the water-soluble whey protein fraction is relatively more heat-stable than that in the meal, indicates that processing conditions required for maximum PE of these fractions may differ from conditions required for the whole meal.

The lower nutritive values of acid-precipitated protein and residue, compared with toasted soybean meal, probably result from an unequal distribution of essential sulfur amino acids caused by fractionation of the meal.

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