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Effects of raw soy flour feeding in weanling pigs: comparison with rats and monkeys

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Abstract. Feeding of raw and heated soy flour (RSF & HSF) for 4 or 8 weeks to weanling swine was compared with feeding of casein diets. There was essentially no growth in pigs fed RSF, whereas body weight increased 4.5 fold over 8 weeks with either casein or HSF. No change in pancreas weight as percent body weight was found in any group in either experiment. Both total pancreatic RNA and DNA were decreased in RSF groups, although RNA and DNA/mg pancreas were increased by the RSF diets. Total pancreatic protein, as well as protein/mg, were decreased in RSF-fed pigs compared to casein and HSF. Pancreatic trypsin, chymotrypsin, lipase, and amylase, and fecal trypsin were decreased in RSF-fed pigs to varying degrees compared to either HSF or casein. HSF and casein performance was equivalent in all parameters measured. Results of these experiments are compared with results of feeding similar diets to rats and monkeys.

Introduction

The effects of raw and heated soy products on growth and pancreatic physiology and acinar cell biochemistry have been studied by numerous investigators in recent years for a number of reasons: (a) RSF produces pancreatic enlargement in some small animal species (rats, guinea pigs, chickens), but not in larger animals [2, 4–7, 14, 15]; (b) raw soy flour has been shown to be both a cocarcinogen and a weak carcinogen of the pancreas in rats [8–9]; (c) soy protein is being used in more and more human products, and (d) pancreatic cancer is increasing in Western countries [10]. The deleterious effects of raw soy on the physiology and biochemistry of pancreata of rodents may or may not have any relevance for larger species, particularly humans.

These studies were undertaken to compare short-term response to soy diets in the rat, with the responses of a primate (rhesus monkey), and the pig, which is both omnivorous and has a digestive tract similar to that of humans. It was hoped that by elucidating the early biochemical responses of the animals we might be able to predict what the long-range pancreatic response

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might be. Effect of soy diets on rats, monkeys, and pigs in four-week experiments have previously been published and will be reviewed here [14]. Diets, materials, and methods have also been detailed in an earlier publication [14]. The four week studies in weanling pigs were extended to eight weeks; results of both four and eight week pig studies will be reported here.

Animals and diets

Weanling female crossbred pigs were housed individually in stainless steel cages (4 week study) or three per pen in facilities at the Ralston Purina Research Farm, Gray Summit, Mo. A standard casein control diet was fed for the first week of the experiments, and either casein, raw soy flour (RSF), or heated soy flour (HSF) diets were fed for the following eight weeks. Diets contained 20% protein, 8% fat, 5.63 ash, 5% moisture, and 2% crude fiber [14]. At necropsy animals were killed by electrocution, bled immediately, and pancreata removed, weighed, and preserved for histological and biochemical analysis as previously described [14]. Assays and chemicals have been previously reported [14].

Statistics. Means were compared using Duncan's multiple range test and analysis of variance [13].

Pancreas and Body Weights

Body weights were not significantly different between pigs fed casein and HSF diets at either four or eight weeks (Table 1). The RSF-fed pigs, however, maintained their original body weight but gained almost nothing. Body weights in RSF-fed pigs at both four and eight weeks were significantly lower than body weights of pigs fed either casein or HSF. The pigs which were fed RSF for eight weeks appeared listless and weak at the end of the experiment, whereas both casein-fed and HSF-fed pigs were vigorous and healthy in appearance. The hematocrit was not measured at necropsy, but appeared to be very low in the RSF group. It is doubtful that these pigs would have survived much longer on the RSF diet, based on their appearance. This is in contradistinction to rats, which have been fed RSF as a sole source of protein successfully for more than a year [8, 9]. Pancreas weight as per cent body weight did not change in any group, regardless of diet.

Pancreatic biochemistry

Pancreatic nucleic acid and protein content were similar at both four and eight weeks in pigs fed casein and HSF (Table 2). Total nucleic acid and protein content was significantly lower in pancreata of RSF-fed pigs, as would be expected, due to lower pancreas weight. However, DNA and RNA were both increased, and protein decreased, when measurements were made

Table 1. Body and pancreas weights in pigs at four and eight weeks.*

		Diet Protein Source		
		Casein	RSF	hSF
Initial body wt, kg**		6.03[a]	6.33[a]	6.46[a]
Final body wt, kg	4 wks	16.26[a]	7.86[c]	14.94[b]
	8 wks	29.90[b]	7.07[a]	29.77[b]
Pancreas wt, mg	4 wks	25.6 [b]	13.7 [b]	27.8 [b]
	8 wks	50.0 [b]	11.9 [a]	54.7 [b]
Pancreas wt as % of body wt	4 wks	0.16[a]	0.18[a]	0.19[a]
	8 wks	0.16[a]	00.17[a]	0.18[a]

*Row means not followed by the same letter are significantly different, $P \leq 0.05$.
**4-week experiment. Initial weights of pigs used in the 8 week study were also between 6 and 6.5 kg.

Table 2. Pancreatic protein and nucleic acids in pigs fed four or eight weeks with casein, heated, or raw soy flour as protein source.*

		Diet Protein		
Total/pancreas, mg	Wks	Casein	RSF	HSF
RNA	4	475.5 ± 61.6[b]	307.0 ± 19.1[a]	564.0 ± 51.0
	8	1499.0 ± 100.0[b]	374.0 ± 48.0[a]	1749.0 ± 96.0[b]
DNA	4	135.9 ± 11.4[b]	106.7 ± 4.8[a]	153.9 ± 27.4[b]
	8	197.0 ± 30.0[b]	103.0 ± 6.0[a]	158.0 ± 11.0[b]
Protein	4	4790.0 ± 450.0[b]	2210.0 ± 90[a]	5100.0 ± 500[b]
	8	8890.0 ± 3500.0[b]	1720.0 ± 190.0[a]	10320.0 ± 5700.0[b]
mg/g dry wt				
RNA	4	73.0 ± 6.3[b]	94.0 ± 4.0[a]	80.5 ± 3.7[b]
	8	120.1 ± 6.1[a]	126.0 ± 8.4[a]	127.9 ± 3.1[a]
DNA	4	22.2 ± 2.0[b]	32.9 ± 1.7[a]	21.5 ± 2.4[b]
	8	15.3 ± 1.6[b]	36.4 ± 2.6[a]	11.7 ± 0.9[b]
Protein	4	743.0 ± 25.0[b]	679.0 ± 20.0[a]	719.0 ± 16.0[b]
	8	716.0 ± 17.9[b]	581.0 ± 25.3[a]	753.0 ± 16.5[b]

*Row means not followed by the same letter are significantly different, $P \leq 0.05$.

on a per gram of pancreas basis in RSF-fed pigs. This may have been due to the poor digestibility of the protein and its nutritional inavailability as well as to the trypsin inhibitor content. Pancreatic trypsin, chymotrypsin, lipase and amylase and fecal trypsin were substantially reduced (Table 3) in pigs fed RSF; no significant differences were found between HSF and casein diets at either 4 or 8 weeks.

These results are in contrast to results we and others have found in rats. In rat work done in this laboratory, both RNA and protein were substantially elevated by feeding RSF, and protein/DNA and RNA/DNA were increased, indicating a hypertrophic response [14]. Although neither the quantity of DNA per milligram pancreas nor total DNA per pancreas was different from controls, the pancreas weight in rats averaged 0.73% of body weight as compared to 0.35–0.45% in controls following 4 weeks of RSF feeding.

Table 3. Pancreatic digestive enzymes and fecal trypsin in pigs fed casein or raw or heated soy protein for 4 or 8 weeks.*

Enzyme, units/mg	Wks	Diet Protein		
		Casein	RSF	HSF
Trypsin	4	129 ± 12[a]	92 ± 6[b]	122 ± 11[a]
	8	157 ± 7[a]	107 ± 15[b]	172 ± 9[a]
Chymotrypsin	4	0.044 ± 0.008[a]	0.009 ± 0.001[b]	0.044 ± 0.005[a]
	8	0.073 ± 0.007[a]	0.018 ± 0.004[b]	0.076 ± 0.006[a]
Lipase	4	–	–	–
	8	19.81 ± 1.89[b]	4.04 ± 0.8[a]	21.9 ± 2.15[b]
Amylase	4	–	–	–
	8	43.5 ± 4.1[b]	4.9 ± 0.8[a]	53.8 ± 5.0[b]
Fecal trypsin	4	7.6 ± 1.2[b]	2.5 ± 0.5[a]	9.7 ± 1.2[b]

*Row means not followed by the same letter are significantly different, $P \leq 0.05$.

There were no histologic observations of cellular enlargement, although pancreata were enlarged approximately 1.8 times normal size. The protein and RNA per milligram pancreas increased only about 1.3 times normal. The increases in RNA and protein indicate hypertropy, despite the lack of histological observations of cellular enlargement. The difference between the increase in RNA and protein, and the increase in pancreas weight indicated that only part of the enlargement was due to a hypertrophic response. When the pancreatic DNA was calculated as a percent of body weight, as is usual for pancreas weight, it was apparent that DNA was increased even after this short feeding period, and the results seen indicated a hyperplastic, as well as hypertrophic response to RSF in the rat. In one four-week experiment [14] (5 male and 5 female rats per diet group) pancreatic DNA, calculated as percent body weight, was as follows: casein, 0.0215 ± 0.0005; RSF, 0.0315 ± 0.0015; isolated soy protein (ISP) 0.0244 ± 0.008. The value for RSF was higher than for either casein or ISP, which were not statistically different from each other ($F = 11.301$, $P \leq 0.005$). (Trypsin inhibitor content of heated ISP and HSF are similar).

In monkeys, no physical pancreatic changes were seen with any of the protein sources, including RSF, over a four-week period [14]. Feed consumption was also similar in all groups. An increase in pancreatic trypsin was noted when RSF was fed, but it was not significant. There were significant changes in two pancreatic enzymes with raw soy protein products: lipase was 1.4 units/mg in RSF-fed monkeys, 11.4 in the casein-fed group, and 28.7 in a group fed ISP. Neither RSF nor HSF lipase was significantly different from the casein-fed group, but the raw and heated soy products were different from each other. Pancreatic amylase was reduced by RSF to 38% of the casein value, but the amylase in unheated ISP-fed monkey pancreata was not significantly altered compared to the casein controls. RSF reduced fecal

trypsin in monkeys as it did in pigs; in contrast, we and others have found fecal trypsin to be increased in rats fed RSF [12, 14].

Results obtained in this laboratory previously [14] have shown that marked changes occur not only in pancreas weight, but in pancreatic biochemical composition of rats fed RSF. Enzymatic and some biochemical changes were also seen in monkeys and pigs fed RSF. All three of these species responded differently to similar RSF diets, (Table 4) indicating that the biological control mechanisms are dissimilar with regard to pancreatic growth and biochemistry. Whether any of these animals is an appropriate model for humans is not known. Rats, albeit a convenient and useful laboratory species, have been demonstrated to have different growth requirements (amino acids, certain vitamins) from humans [1, 3, 11], and a different growth pattern. They are fast-growing, fur-bearing, and short-lived, among other things. Swine have been used as models for humans due to the similarity of digestive tracts. Again, although they have omnivorous feeding habits, there are major differences, the most obvious being the rapid rate of growth, size at maturity, and life span. Even other primates, although they should be substantially better models than rats or pigs due to closer similarity in growth rate, life span, reproductive similarity, digestion, dentition, etc. to humans than the other species used here, are not ideal. In addition to being fur-bearing and largely vegetarian by habit, these monkeys, for example, had a fecal transit time of approximately 5–6 hours [14]; this is substantially less than fecal transit time in humans.

Table 4. Summary: Effect of feeding raw soy flour to rats, pigs, or monkeys compared to a casein or heated soy control diet in 4 week experiments [14].*

Parameter	Rat	Pig	Monkey
Growth	−60%	−84%	NC
Feed consumption	−10%	−45%	NC
Fecal trypsin	+300 + 400%	−48%	−50%
Nitrogen digestibility	−5%	−30 − 40%	−9%
Pancreas wt % body wt	+50%	NC	NC
Pancreatic: DNA/mg	NC	+30%	NC
RNA/mg	+40%	+20%	NC
Protein/mg	+47%	−7%	NC
Total DNA	NC	−25%	NC
Total RNA	+30%	−40%	NC
Total protein	+33%	−60%	NC
Protein/DNA	+40%	−40%	NC
Protein/RNA	NC	−25%	NC
RNA/DNA	+20 + 40%	−20 − 25%	NC
Enzymes: Units/mg pancreas			
Trypsin	NC	−30%	+35%
Chymotrypsin	NC	−80%	−40%
Lipase	−88%	−83%	−80%
Amylase	−68%	−93%	−60%

*NC: no change.

Despite the differences noted, when rats, swine, or monkeys were fed HSF, the growth rates and biochemical parameters measured were not different from rats, swine, or monkeys fed the casein control diet. The choice of an animal model in this case is much less important than the choice of an appropriate protein model.

RSF is a poor feedstuff. Legume seeds, including soy beans, are well protected from foraging animals by trypsin and chymotrypsin inhibitors, lectins, and other antimetabolites and toxins including occasional cyanogens [6, 7]. Long years of human experience with these materials have demonstrated that cooking of peas, beans, grams, lentils, and soybeans renders them both edible and nutritious. In the course of these studies, we procured 'recipes' for soy milk of the type used in China for decades from two of our Chinese colleagues. Both called for soaking the beans overnight, draining the soak water, and boiling the ground soybeans for at least an hour, thereby inactivating most of the trypsin inhibitor. Proper cooking was known to be essential to making good soy milk, and cooking or heating is essential to human consumption of all soya products and other legumes both for safety and nutritional quality.

The common factor for each of the species studied in this research, and surely for humans as well, is proper heating of soy protein products to destroy trypsin inhibitors and other antinutritional factors. Such soy products are an excellent source of dietary protein for both humans and various species of animals.

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