Effect of variable protein contents in diets containing *Phaseolus vulgaris* beans on performance, organ weights and blood variables in piglets, rats and chickens

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A comparison was made of the effects of antinutritional factors present in *Phaseolus vulgaris* on piglets, rats and chickens. Also the hypothesis of whether the negative effect on weight gain due to the inclusion of raw *Phaseolus vulgaris* in the diet can be attributed to an insufficient supply of amino acids was tested. Test diets containing 200 g raw *Phaseolus* beans/kg were balanced for digestible protein and amino acids; in one diet extra casein was incorporated. The main response criteria were live-weight gain and the weight of various organs including the intestine. Live-weight gain in piglets was markedly reduced during feeding 200 g raw *Phaseolus vulgaris*/kg in the diet, but not in rats and chickens. Addition of casein did not improve the weight gain of the piglets, indicating that a toxic factor was responsible for the reduced weight gain and not an insufficient supply of amino acids. The weights of the spleen and thymus were markedly reduced in the piglets when the diets with raw *Phaseolus* beans were given, but not in the rats and chickens. Additional supply of casein did not change this effect. Indications were found that when the supply of dietary protein is adequate there is no reduction in pancreas weight with raw *Phaseolus* beans as was observed in previous experiments. The weight of the intestine was increased in all three species due to feeding raw *Phaseolus vulgaris*.

Antinutritional factors: Lectins: *Phaseolus vulgaris*: Chicken: Piglet: Rat

Phaseolus vulgaris beans contain various antinutritional factors (ANF) (Bressani, 1983). The main ANF in these beans are lectins, trypsin inhibitors and in the coloured flowering varieties, tannins. It is known that the lectins in *Phaseolus vulgaris* are highly toxic and are the main factors responsible for the negative effects when these beans are fed to livestock (Pusztai, 1985).

Huisman *et al.* (1990) found that piglets were more sensitive to ANF in *Phaseolus vulgaris* than rats. Differences between the two animal species were noted in effects on live-weight gain and feed conversion ratio. Weights of pancreas and spleen in these piglets were markedly reduced when raw *Phaseolus* beans were given, but in rats there were no such effects. In that study nitrogen digestibility of the diets containing 200 g raw *Phaseolus* beans/kg was 47·6% in piglets and 64·5% in rats. As a result less digestible amino acids were available for absorption in piglets than in rats. A hypothesis can, therefore, be that the former marked negative effect in piglets compared with rats, may be related to an inadequacy of protein and amino acids. This hypothesis was checked in the present study.

A control and three *Phaseolus* bean-containing diets were balanced for digestible protein

and amino acids using digestibility coefficients for piglets. In one diet extra casein was included to increase the protein and amino acid supply. These diets were given to piglets, rats and also to chickens. Chickens were incorporated in the study because they are important farm animals.

The objectives of the study were: (1) comparison of effects of ANF present in *Phaseolus vulgaris* in piglets, rats and chickens; (2) testing the hypothesis that the negative effects of inclusion of raw *Phaseolus vulgaris* on live-weight gain is due to an insufficient protein and amino acid supply; (3) testing whether the weight of the pancreas in piglets is affected by inclusion of raw *Phaseolus vulgaris* in diets balanced for digestible protein and amino acids.

MATERIALS AND METHODS

Four diets were formulated (Table 1) according to the following scheme. 1, Control (C); 2, test diet containing 200 g raw *Phaseolus vulgaris* beans/kg and additional casein (R0); 3, test diet containing 200 g raw *Phaseolus vulgaris* beans/kg (R60); 4, test diet containing 200 g/kg *Phaseolus vulgaris* beans/kg, toasted for 40 min (T).

The main protein source in diet C was casein. Casein and fish were chosen as protein sources to ensure that no ANF were present in the control diet. In the test diets, part of the casein was replaced by the *Phaseolus* beans. The beans were from a commercial mixed batch. A portion of this batch of beans was steam-heated for 40 min. Before heating, the beans were cracked in a hammermill. The composition of the beans is given in Table 2. Diets R0 and R60 were formulated with raw beans. For diet R0 the digestibility of the raw bean protein was assumed to be 0%. This means that in this diet as much casein was included as in the control diet.

In a separate digestibility trial with piglets the faecal digestibility of N of the batch of beans heated for 40 min (T) was found to be approximately 60%. This digestibility coefficient was used to balance the bean diets R60 and T on the basis of their content of digestible protein (N × 6·25). The amounts of amino acids in the piglet diets were calculated using ILOB tables (E. J. van Weerden, J. Huisman and Van Leeuwen, unpublished results) for pigs. The amounts of digestible amino acids in the beans were calculated from the same table with a correction for the lower protein digestibility assuming that the digestibility of the amino acids was reduced in proportion to the reduction for the protein. The rats were fed on the piglet diets. The amounts of amino acids in the chicken diets were based on the values published in the CVB (1988) table.

Free lysine, methionine, threonine and tryptophan were included to balance the diets for these amino acids. In the chicken diet extra methionine and arginine were included. As indicated in the scheme an extra treatment (R0) was incorporated. The diets were pelleted without steam, the pellet size was 3 mm for the piglets and the chickens and 9 mm for the rats. The composition of the diets is given in Table 1.

Animals, experimental scheme and management of the experiment

Twelve piglets, fifteen rats and sixty chickens were each given each diet. The piglets were of the crossbred Dutch Landrace × Dutch Yorkshire type. They were housed in metabolism cages with two in each cage. The piglets were assigned to the treatments in such a way that the mean live weight and the variation per treatment were about the same for each treatment. The rats were Wistar type; they were weaned at 4 weeks of age and immediately placed in the metabolism cages. The procedure for adaptation of piglets and rats to the diets, the housing conditions and the method for assignment to the treatments have been

Table 1. *Composition of the diets*

Ingredients (g/kg)	Diet C	Diet R0	Diet R60 and T
Phaseolus vulgaris beans	—	200	200
Casein	131	131	98
Fish meal	50	50	50
Maize starch	556	350	393
Wheat bran	50	50	50
Cellulose	21	6	6
Soya-bean oil	29	58	46
D-Glucose	60	60	60
Sugar-cane molasses	40	40	40
Vitamin–mineral mixture*	10	10	10
$CaCO_3$	2	4	3
$CaHPO_4.2H_20$	29	25	26
$CaCl_2$	3	3	3
$NaHCO_3$	12	12	12
$KHCO_3$	6	—	0·1
Amino acids added to the diets of the piglets and rats			
L-Lysine	—	—	1
DL-Methionine	1·1	1·1	1·6
L-Threonine	0·6	0·6	0·8
L-Tryptophan	0·2	0·2	0·3
L-Arginine			
Amino acids added to the diets of the chickens†			
L-Lysine	0·2	0·1	1·1
DL-Methionine	3·7	2·7	3·6
L-Threonine	0·6	0·6	0·8
L-Tryptophan	0·2	0·2	0·3
L-Arginine	6·2	6·0	5·5
Calculated contents g/kg			
Crude protein (nitrogen × 6·25)	168	210	184
Digestible crude protein	155	155	155
Ash	60	64	64
Crude fat	41	74	60
Crude fibre	25	25	25
Calcium	96	96	96
Phosphorus	80	80	80
Digestible lysine			
Piglets and rats‡	127	127	127
Chickens	126	147	128
Digestible methionine and cystine			
Piglets and rats‡	65	65	65
Chickens	87	84	85
Digestible threonine			
Piglets and rats‡	73	73	73
Chickens	72	84	73
Digestible tryptophan			
Piglets and rats‡	21	21	21
Chickens	20	24	21

* Supplies (/kg feed): retinol 2·7 mg, cholecalciferol 45 μg, DL-α-tocopherol 40 mg, menadione 3 mg, riboflavin 5 mg, nicotinic acid 30 mg, D-pantothenic acid 15 mg, choline chloride 120 mg, cyanocobalamin 40 μg, ascorbic acid 50 mg, $CuSO_4.5H_2O$ 20 mg, $ZnSO_4.H_2O$ 200 mg, MnO 70 mg, $FeSO_4.7H_2O$ 400 mg, $CoSO_4.7H_2O$ 2·5 mg, $Na_2SeO_3.5H_2O$ 0·2 mg, KI 0·5 mg.

† The extra amino acids were substituted for maize starch.

‡ Based on digestibility values from piglets.

Table 2. *Chemical composition (g/kg) and antinutritional factor contents in the* Phaseolus vulgaris *bean*

	Unheated beans	Heated beans
Dry matter	894	897
Ash	49	50
Crude protein (nitrogen × 6·25)	224	232
Crude fat	20	20
Crude fibre	71	65
N-free extract	530	529
Haemaglutinins (HA)*	40	0·8
Trypsin inhibitors (mg)†	4·7	0·3

* Haemagglutination of rabbit erythrocyte cells. Haemagglutination activity (HA) is expressed in units/mg sample. One unit is defined as the smallest amount of sample necessary for agglutination under test conditions.
† mg inhibited trypsin/g product.

described previously (Huisman *et al.*, 1990). The chickens, 1-day old Hybro birds, were placed in battery cages with ten birds in each cage. For the first 2 d they received a normal commercial starter diet. During the next 4 d the control chickens were adapted to diet C and the test chickens to a diet containing 200 g commercial toasted batch of *Phaseolus* beans/kg. During adaptation the chickens were fed according to the following scheme: day 1, 750 g practical diet and 250 g commercial toasted bean diet/kg; day 2, 500 g practical diet and 500 g commercial toasted bean diet/kg; day 3, 250 g practical diet and 750 g commercial toasted bean diet/kg, day 4, 1000 g commercial toasted bean diet/kg. At day 5 the chickens were changed to the specially prepared test diets, and the control chickens remained on the control diet.

All three species were fed on a restricted basis according to a scheme based on 2·2 times maintenance requirement for energy. The daily feed offered for the rats and piglets was calculated according to the following formula: $420 \times 2{\cdot}2/13\,560$, in which 420 is the metabolizable energy required for maintenance (kJ)/unit metabolic-weight (kg body-weight $(W)^{0{\cdot}75}$) and 13 560 is the metabolizable energy (kJ)/kg feed. For the chickens the following formula was used: $520 \times 2{\cdot}2/13\,800$, in which 520 is the metabolizable energy for maintenance (kJ/kg $W^{0{\cdot}75}$) and 13 800 is the metabolizable energy (kJ)/kg feed.

Body-weight was measured weekly and the feeding schedule was adjusted twice weekly based on the expected growth. Weight gain of the piglets was measured over a period of 2 weeks. This time-period was chosen arbitrarily because in a previous experiment piglets lost weight after feeding raw *Phaseolus* beans for 3 weeks. The weight gain of the rats and the chickens was measured over 3 weeks.

Collection of organs and blood samples

On the day following the end of the growth period, from each of the treatments C, R60 and T, seven piglets, seven rats and twelve chickens were taken at random for dissection and collection of the various organs and the intestine. From the piglets of treatment R0 all twelve animals were dissected. Just before dissection the animals were weighed. All animals were then anaesthetized using Fluothane®, nitrous oxide and oxygen. After anaesthesia the abdomen was opened and the organs and intestine were removed quickly and weighed immediately. The content of the intestine was removed by hand by stripping. The weights of the organs and intestine are expressed relative to body-weight.

Chemical analyses

The dry matter (DM) content was determined by drying the samples to constant weight at 101°. Ash was determined by incineration at 550° during 4 h. N was analysed in fresh material using a Technicon AutoAnalyzer. After wet digestion with 2·0 M-potassium sulphate solution in the 18 M-sulphuric acid and selenium as catalyst, the N was bound by hypochlorite and phenol. This N complex was measured at 630 nm. Crude fat was analysed by treating with 3 M-hydrochloric acid for 1 h and drying for 3 h under vacuum at 100°, followed by 8 h extraction with diethyl ether. Crude fibre was determined according to NEN 3326 (1966). After boiling the sample with a sulphuric acid solution of standard concentration the residue was boiled with sodium hydroxide solution of a standard concentration. The insoluble residue was then separated, washed, dried and weighed and the loss in mass determined on combustion.

Urea content in blood plasma was measured according to the patented two-step American Monitor urea assay (US patent 4.074.972). The primary reaction occurs between the phthalaldehyde compound and urea resulting in the formation of an isoindoline derivative. The isoindoline derivative then reacts with 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline to form an intensely coloured product which can be quantified by photometric measurement at 510 nm.

N-free extract was calculated as DM − (ash + (N × 6·25) + crude fat + crude fibre).

The content of trypsin inhibitors were analysed according to Kakade *et al.* (1974). The content of haemagglutinins was measured according to Valdebouze *et al.* (1980), with modifications.

Statistical analysis

The values for the different criteria are given as means and standard deviations. The differences between treatments were analysed by Student's *t* test.

RESULTS

Weight gain, feed intake and feed conversion ratio

The results of weight gain, feed intake and feed conversion ratio of the three animal species are presented in Table 3. In piglets there was weight loss with both diets R0 and R60. The weight loss on diet R0, in which extra casein was incorporated, was similar to that of the treatment without casein. In rats, weight gains with diets R0 and R60 were almost similar to that with diet C. In chickens the weight gain with diet R60 was significantly reduced (9%) compared with the control. When diet R0 (containing extra casein) was given, weight gain in chickens was ($P < 0{\cdot}05$) above that of the control animals.

When diet T was given the relative weight gain in the piglets was nearly at the control level and slightly above that of rats and chickens. Feed intake was reduced drastically in the piglets when diets R0 and R60 were given. The extra casein in diet R0 had no positive effect on feed intake. In rats and chickens the feed intake was either slightly reduced or not reduced during feeding diets R0 and R60. When diet T was given, feed intake in piglets was slightly reduced compared with diet C, and in the rats and chickens feed intake was not reduced.

Effects of the same magnitude were found for feed conversion ratio.

Organs and blood indices

Values for organ weights are presented in Tables 4, 5 and 6. The values for the weight of the small intestine are given in Table 7 and those on plasma contents of urea in Table 8.

Table 3. *Weight gain*, feed intake* and feed conversion ratio* in piglets, rats and chickens given diets containing raw (R0, R60) and toasted (T)* Phaseolus vulgaris *beans*

(Mean values and standard deviations)

Dietary treatments	Gain (g/d) Mean	SD	Feed intake (g/d) Mean	SD	Feed conversion ratio Mean	SD
			Piglets			
C	151·6[a]	32·9	233·9[a]	53·9	1·55[a]	0·09
R0	−2·1[b]	26·3	146·0[b]	52·8	Negative	
R60	−3·5[b]	23·7	134·8[b]	103·0	Negative	
T	145·8[a]	37·0	225·4[a]	43·3	1·56[a]	0·17
			Rats			
C	3·0[a]	0·6	12·3[a]	1·5	4·19[a]	0·47
R0	3·1[a]	0·4	12·5[a]	1·3	4·03[a]	0·30
R60	2·9[a]	0·5	12·3[a]	1·2	4·26[a]	0·37
T	3·1[a]	0·4	12·4[a]	1·2	3·97[a]	0·33
			Chickens			
C	11·7[a]	0·7	20·8[a]	0·4	1·78[a]	0·09
R0	12·1[b]	0·3	20·8[a]	0·4	1·72[b]	0·05
R60	10·6[c]	0·4	20·0[a]	0·2	1·89[c]	0·05
T	11·9[a]	0·5	20·9[a]	0·3	1·76[ab]	0·05

R0, unheated beans + extra casein; R60, unheated beans; T, toasted beans.

[a,b,c] Values with different superscript letters in the same column of each animal species were significantly different ($P < 0·05$).

* In piglets measured over 14 d and in rats and chickens over 21 d.

Table 4. *Weight of organs in piglets (g/kg body-weight) given diets containing raw (R0, R60) and toasted (T)* Phaseolus vulgaris *beans*

(Mean values and standard deviations)

Dietary treatment	Liver Mean	SD	Pancreas Mean	SD	Kidney Mean	SD	Spleen Mean	SD	Thymus Mean	SD
C	24·4[a]	2·5	1·8[a]	0·3	4·8[a]	0·8	2·7[a]	0·9	3·7[a]	1·2
R0	28·6[bc]	3·6	1·6[a]	0·2	5·0[a]	0·5	1·5[b]	0·4	1·8[b]	0·7
R60	24·8[a]	5·5	1·6[a]	0·4	4·6[a]	0·7	1·5[b]	0·4	1·8[b]	1·0
T	27·7[ac]	3·0	1·6[a]	0·2	4·9[a]	0·4	2·1[c]	0·9	3·0[a]	0·7

R0, unheated beans + extra casein; R60, unheated beans; T, toasted beans.

[a,b,c] Values with different superscript letters in the same column were significantly different ($P < 0·05$).

The liver weight of the piglets fed on diet R0 was significantly ($P < 0·05$) higher than those for diet C and R60. The liver weights were not significantly different between the treatments in the rats. The liver weight of the chickens fed on diets R60 and T were lower than those of the control animals; the difference between diet C and diet T was significant ($P < 0·05$).

The pancreas weights of piglets did not differ significantly between treatments. The pancreas weights of the rats given diets R60 and T were slightly increased. In chickens fed on raw beans the pancreas weight was significantly ($P < 0·05$) higher when compared with that of the control group. The pancreas weight of the chickens fed on diet T did not differ from control values.

Table 5. *Weight of organs in rats (g/kg body-weight) given diets containing raw (R60) and toasted (T)* Phaseolus vulgaris *beans*

(Mean values and standard deviations)

Dietary treatment	Liver		Pancreas		Kidney		Spleen		Thymus	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	42·3	7·2	5·1	1·5	8·7	0·5	2·4	0·3	3·5	1·1
R60	42·6	7·9	6·9	2·3	8·4	0·3	2·3	0·3	3·3	0·9
T	44·7	10·1	6·5	2·2	8·5	0·3	2·5	0·3	3·7	0·3

Values were not significantly different.

Table 6. *Weight of organs in chickens (g/kg body-weight) given diets containing raw (R60) and toasted (T)* Phaseolus vulgaris *beans*

(Mean values and standard deviations)

Dietary treatment	Liver		Pancreas		Kidney		Spleen		Thymus		Bursa fabricius	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	30·3^{a}	2·9	2·9^{a}	0·4	10·2^{a}	1·3	1·0^{a}	0·2	5·5^{a}	0·9	3·4^{a}	0·9
R60	28·3^{ab}	3·1	3·9^{b}	0·5	11·7^{b}	0·7	1·0^{a}	0·3	5·1^{ab}	0·9	3·7^{ab}	1·0
T	26·4^{b}	3·3	3·0^{a}	0·3	10·2^{a}	1·0	1·1^{a}	0·3	4·7^{b}	0·7	4·3^{b}	0·8

^{a,b} Values with different superscript letters in the same column were significantly different ($P < 0·05$).

Table 7. *Weight of intestine in piglets, rats and chickens (g/kg body-weight) given diets containing raw (R60) and toasted (T)* Phaseolus vulgaris *beans*

(Mean values with standard deviations)

Dietary treatment	Small intestine						Large intestine	
	Piglets		Rats		Chickens		Chickens	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	35·2^{a}	5·0	24·3^{a}	4·2	22·4^{a}	2·2	5·7^{a}	1·9
R60	47·0^{b}	5·6	40·0^{b}	4·2	34·8^{b}	4·9	11·2^{b}	4·4
T	39·2^{a}	3·9	24·0^{a}	2·0	19·5^{c}	2·4	5·5^{a}	2·7

R60, unheated beans; T, toasted beans.

^{a,b,c} Values with different superscript letters in the same column were significantly different ($P < 0·05$).

In piglets and rats there were no significant differences in kidney weight between the treatments. In chickens the kidney weight of diet R60-fed birds was significantly higher than that of the controls ($P < 0·05$).

The spleen weight of the piglets fed on diet R60 was significantly lower ($P < 0·05$) than that of the control and diet T-fed animals. In the rats and chickens there were no significant differences between the treatments.

In the piglets the weight of the thymus was markedly ($P < 0·05$) reduced when diets R0 and R60 were given. In rats no significant differences between the treatments were found

Table 8. *Urea contents (mg/l) in plasma of piglets and chickens given diets containing raw (R0, R60) and toasted (T)* Phaseolus vulgaris *beans*

(Mean values and standard deviations)

Dietary treatment	Piglets		Chickens	
	Mean	SD	Mean	SD
C	80[a]	25	12[a]	1·88
R0	473[b]	207	—	—
R60	455[b]	101	10[a]	2·7
T	126[a]	58	10[a]	2·7

R0, unheated beans + extra casein; R60, unheated beans; T, toasted beans.
[a,b] Values with different superscript letters in the same column were significantly different ($P < 0·05$).

($P < 0·05$). In chickens the weight of the thymus with diet T was significantly lower compared with the control.

The bursa fabricius of the chickens fed on diet T were heavier ($P < 0·05$) compared with those of the control birds. In all three species the weight of the small intestine with diet R60 was higher ($P < 0·05$) than that with diets C and T. In chickens the weight of the large intestine was determined, and on diet R60 was found to be double ($P < 0·05$) that on diets C and T.

From piglets and chickens blood samples were taken for the determination of urea. In piglets fed on diets R0 and R60 the plasma urea content was about five times higher than the diet C-fed animals, while the content in the samples from the diet T-fed piglets was not significantly increased. In chickens there were no significant differences between the treatments (Table 8).

DISCUSSION

Inclusion of 200 g *Phaseolus* beans/kg diet caused a markedly reduced feed intake and weight gain in piglets, but little or no effect in rats and chickens. Also the weight of the spleen and the thymus was distinctly reduced in piglets, but not in rats and chickens. These results indicate that the piglet was much more sensitive to ANF present in the *Phaseolus* bean than the rat and the chicken. The rat and the chicken seemed similar in sensitivity.

The fact that the spleen and thymus weights were only affected in the piglet may be an indication that the immune system of the piglet is more sensitive to factors present in *Phaseolus* beans than the rat and the chicken. It is difficult to explain which factors are responsible for effects on the immune system, although it is known that lectins (Pusztai, 1989) affect the immune system.

In all three animal species the weight of the small intestine fed on diet R60 was significantly increased compared with the controls, indicating that the small intestinal tissue of all three animal species is sensitive to the ANF present in this bean. De Oliviera *et al.* (1988) found that the enhanced weight of the small intestine in rats when feeding raw *Phaseolus* beans, is associated with lectins.

As described previously the diets were balanced for digestible protein and amino acids and, moreover, in diet R0 extra casein was incorporated. In spite of balancing the diets the piglets fed on the raw *Phaseolus* beans did not gain weight. The values for the plasma urea contents in the piglets indicate that protein deposition was seriously reduced. This disturbance in protein deposition cannot be attributed to an insufficient amino acid supply and, therefore, another factor must be responsible for the negative effects.

Similar indications were found by King *et al.* (1983). They showed that when pigs were given the same amount of a control diet or a *Phaseolus* bean-containing diet the weight gain in the bean-fed pigs was markedly reduced. In a study with rats by Pusztai *et al.* (1981) it was demonstrated that lectins are responsible for the reduction in feed intake and weight gain when *Phaseolus* beans are given.

Huisman *et al.* (1990) found a reduced pancreas weight in piglets when diets contained 200 g raw *Phaseolus* beans/kg. The N digestibility of the raw bean diet in that study was low compared with the control diet (47·6 *v.* 84·7 %), and as a result the supply of the amino acids was inadequate from the bean diet. In the studies of Green *et al.* (1986), Gumbmann *et al.* (1986), and Liener *et al.* (1985) it was shown that pancreas weight relative to body-weight was positively correlated with the protein content in the diet. Moreover, Green *et al.* (1986) demonstrated that pancreas growth was inhibited by insufficiency of essential amino acids. In the present experiment the diets, which were balanced for digestible protein and amino acids, did not result in a difference in the weight of the pancreas of the piglets. These results indicate that when the supply of protein is adequate there is no reducing effect on the weight of the pancreas of piglets due to inclusion of *Phaseolus* beans in the diet.

A striking observation is that feeding raw *Phaseolus* beans led to pancreatic hypertrophy and increased small intestine weight in rats and chickens, with little or no effect on weight gain. It seems that under conditions of the present study (diets balanced for digestible protein and amino acids, energy, vitamins, minerals and low feeding level) these animals are able to compensate for effects on the pancreas and the gut wall.

Hypertrophy is indicative of increased activity of the pancreas resulting in increased enzyme secretion (Liener & Kakade, 1980). These enzymes are rich in S-containing amino acids (Liener & Kakade, 1980). Reports by Barnes *et al.* (1962), Borchers (1961, 1962), Khayambashi & Lyman (1966), show that addition of extra methionine, threonine and valine to the diet can eliminate the negative effect of trypsin inhibitors on weight gain.

Under the conditions of the present study the amount of amino acids in the diet should have been sufficient to avoid the effect of stimulated pancreatic activity on weight gain of rats and chickens. The results demonstrate that biological response like pancreatic hypertrophy and increased weight of the small intestine are not always reflected in weight gain.

From the present study it can be concluded that the piglet is distinctly more sensitive to ANF in *Phaseolus* beans than rats and chickens. The marked effects in piglets cannot be explained by inadequacy of amino acid supply.

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