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# The effect of raw soya beans upon the digestion of proteins and upon the function of the pancreas of intact chickens and of chickens with ileostomies

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Osborne & Mendel (1917) showed that raw soya beans (RS) had a low nutritive value for rats, and that cooking them for 3 h greatly increased this value. They attributed this increase in nutritive value to an increase in food consumption and to improved nitrogen absorption.

These findings were confirmed by Hayward, Steenbock & Bohstedt (1936). They showed that for the first 3 days the food consumption of rats was similar on the RS and heated soya-bean (HS) diets. Later the consumption of the HS diet became greater than that of the RS diet. From these observations they concluded that the low nutritive value of the RS diet was not due to unpalatability but was caused by the 'unavailability of some essential protein fraction'; and that application of heat to the RS made this fraction available for absorption and metabolic use.

Hayward & Hafner (1941) showed that addition of cystine or methionine, or of the combination of these two amino acids, improved the growth of rats or chicks given RS diets. Growth was even better when HS diets were thus supplemented. These investigators concluded that cooking not only made the sulphur amino acids available, but in addition, 'the heat treatment possibly increased the availability of the entire protein fraction of the soybeans'.

Additional information on this problem was furnished by Bouthilet, Hunter, Luhman, Ambrose & Lepkovsky (1950), who showed that there was more methionine and more total N in the faeces of chickens with colostomies on RS diets than of those on HS diets, indicating the presence in the faeces of a complex of protein or methionine resisting digestion. The decrease in the digestibility of the RS protein could have been the result of (1) an unavailable protein fraction in the RS, or (2) the conversion of a part of the dietary and endogenous proteins in the intestine into an unavailable protein fraction by the action of the RS. Both these possibilities could operate in reducing the digestibility of the protein.

Previously Ham & Sandstedt (1944) and Bowman (1944) had shown that RS contains a trypsin inhibitor that could inhibit the proteolytic action of trypsin. This information was used in attempts to explain the low nutritive value of the RS. The results were conflicting (Liener, 1958), particularly those of Westfall, Bosshardt &

Barnes (1948) which showed that preparations of crude soya-bean trypsin inhibitor depressed the growth of mice given hydrolysed protein.

Chernick, Lepkovsky & Chaikoff (1948) showed that the pancreases of the chickens fed on the RS diet hypertrophied and contained more proteases. Since then the RS diet has been used for many years in this laboratory to study pancreatic function. This paper presents the results of experiments in which measurements were made of the nitrogen and proteases of the pancreases and of the corresponding intestinal contents at fasting and after eating, before and after adaptation to these diets. It was found that the protease activity of the pancreas was highest at fasting, but that it almost invariably decreased after eating. The decrease represents the algebraic sum of the proteases lost and those synthesized during the feeding period. The enzymes in the contents of the intestine, caecums and colon represent (1) the proteases secreted into the intestine less those that are inactivated, (2) the enzymes lost with the passage of the faeces, and (3) the proteases that are synthesized or destroyed by the intestinal microflora.

The object of the experiments with ileostomized chickens was to distinguish between the effects of these factors on protease activity. The ileostomy made it possible (1) to collect quantitatively the intestinal contents including the total secretion of pancreatic juice before they passed into the colon (the term 'faeces' used later in the text denotes contents so collected); (2) to measure 'faecal' and urinary nitrogen separately, thus permitting the digestibility of the ingested protein to be estimated; (3) to compare the amounts of the proteases in the pancreas with the amounts secreted into the intestine for any desired period of time; (4) to analyse separately the first and later secretions of pancreatic juice after a meal; (5) to observe the changes in the proteolytic activity of the intestinal contents without the complications of the effects that might be caused by the intestinal microflora. In support of this contention, Lepkovsky, Wagner, Furuta, Ozone & Koike (1964) have reported the results of experiments in which the microbial activity of the contents of the small intestine, colon and caecums was assessed by comparing the proportions of nitrogen in the intestinal contents of germ-free and conventional chicks. They concluded that in the conventional chicks, although the contents of the caecums possessed considerable microbial activity, the contents of the small intestine and colon possessed little. This microbial activity was reflected in the behaviour of the proteases in the contents of the different segments of the gastro-intestinal tract. Little or no difference between conventional and germ-free chickens was found in the amount of the proteases in the contents of the small intestine and colon; however, smaller amounts of proteases were found in the caecal contents of conventional chickens than in those of germ-free chickens, suggesting that the inactivation of the proteases was caused by microbial activity.

## EXPERIMENTAL

*Chickens and procedure.* Single Comb White Leghorn chickens of the University of California flock were maintained on UC stock mash (Lepkovsky & Furuta, 1960), which contained 10% commercial extracted, heat-processed soya-bean meal, until they weighed 400–600 g (Expts 1, 2, 3 and 5). They were then transferred to the HS

diets for different periods of time before use. Chickens in Expt 4 weighed 900–1200 g when they were transferred from the UC stock diet to the HS diet.

The intact chickens were kept in groups in wire-screen cages. All chickens with ileostomies were kept in individual wire-screen cages; food consumption was recorded daily and 'faeces' were collected from the cups of the ileostomies every 8 h and refrigerated; they were pooled every 24 h. The urine was collected in plastic sacks by the method of Ariyoshi & Morimoto (1956).

The basic experimental diet contained (g/100 g): soya beans (either raw or autoclaved for 10 min at 15 lb pressure) 60, glucose (Cerelose; Corn Products Co., Argo, Ill., USA) 37·5, $CaCO_3$ 1·0, $Ca_3(PO_4)_2$ 1·0, NaCl 0·5, and Vifort 1·0 ml/kg. (Vifort is a commercial vitamin concentrate made by Endo Products, Richmond Hill, New York, USA, containing per 0·6 ml: vitamin A 5000 USP units, vitamin D 1000 USP units, vitamin C 50 mg, thiamine 1·5 mg, riboflavine 1·2 mg, nicotinamide 10 mg, vitamin $B_6$ 1 mg, and D-panthenol 3 mg). The RS and HS diets were given with and without 0·5 % methionine.

The experimental diets used in Expts 1, 2 and 3 were: HS, HS + methionine, RS and RS + methionine. Some chickens received one meal of any of these diets, having been previously on diet HS. They were then killed. These were the unadapted chickens. Other chickens were similarly treated, but had received for 6 weeks the diet they received at their last meal. These were the adapted chickens.

When both the unadapted and adapted chickens were included in one experiment and were autopsied at the same time, one group of fasting chickens maintained on the HS diet and one group offered the HS diet acted as the control groups for both the unadapted and adapted chickens. Five experiments are reported here.

*Expt* 1. *Intact unadapted and adapted chickens.* Thirty-six chickens were given the control HS diet for 6 weeks. They were starved for from 14 to 16 h, at which time they were divided into twelve groups of three chickens/group. Three groups were killed for autopsy as fasted controls. The rest were treated as follows: three groups were offered one meal of the control HS diet; two groups received similarly the HS diet + methionine, two groups the RS diet, and two groups the RS diet + methionine. After a 5 h feeding period these were killed and examined. These were the unadapted chickens.

At the same time, six groups of three chickens/group were given the HS diet + methionine; six groups the RS diet; and six groups the RS diet + methionine. These were fed for 6 weeks. From the six groups given the HS diet + methionine, three fasted subgroups were killed for autopsy, and three subgroups were offered the diet for 5 h and were then killed and examined. This was repeated for the RS diet and for the RS diet + methionine. These were the adapted chickens.

For examination the chickens were anaesthetized with pentobarbital (Nembutal; Abbott Laboratories Ltd) and decapitated to drain the blood. The pancreases of the three chickens constituting each subgroup were pooled and frozen. Each small intestine was divided into an upper and a lower half, and the contents of each half from three chickens were pooled and frozen. The caecal and colonic contents were similarly treated. All samples were freeze-dried and analysed for proteases and N.

*Expt 2. Unadapted and adapted chickens with ileostomies.* An experiment similar to Expt 1 was done with chickens with ileostomies. After the chickens had been on the diets for 6 weeks ileostomies were performed. When they recovered sufficiently and were eating well, 24 h collections of the 'faeces' were made to secure the 24 h secretion of pancreatic juice, and then all the chickens were denied food for 18 h. Those on the control HS diet were divided into five groups of five; one fasting group was starved for an additional 5 h (total 23 h); the remaining four groups were offered one meal each of the diets for 5 h as follows: one group the HS diet, one the HS diet + methionine, one the RS diet, and one the RS diet + methionine, as in Expt 1. These were the unadapted chickens.

The adapted chickens were treated as follows. All were denied food for 18 h. Food was withheld from one-half of each group on each of the three experimental diets for another 5 h (total 23 h); these acted as the fasting groups. The other half were offered the diet they were eating. At the end of the 5 h feeding period all the starved and fed chickens were killed and examined. The 'faeces' excreted through the ileostomy during the 5 h feeding period were combined with the contents remaining in the intestine, and the material from each chicken was then separately frozen and freeze-dried. The pancreases were removed, then separately frozen and freeze-dried. The material collected was analysed for N and proteases.

*Expt 3. Unadapted chickens with ileostomies.* Chickens 5 weeks of age were given the HS diet for 6 weeks, then ileostomies were performed. After a 4- or 5-day recovery period, two 24 h collections of 'faeces' were made for analysis, after which the chickens were fasted for 18 h. One group of five chickens was killed and examined and acted as the 18 h fasted group. One group of five was fasted for another 5 h and acted as the 23 h fasted group. Four groups of five chickens were offered the following diets for 5 h: one group the control HS diet, one the HS diet + methionine, one the RS diet, and one the RS diet + methionine. The 'faeces' that passed out of the intestines of the chickens during the 5 h feeding period and the contents remaining in the intestines were separately collected and freeze-dried and were separately analysed for N and proteases. The pancreases were collected and similarly treated.

*Expt 4. Chickens with ileostomies: digestibility of nitrogenous and non-nitrogenous fractions of diet.* Chickens weighing 900–1200 g were used to study the digestibility of the nitrogenous and non-nitrogenous fractions of HS and RS diets. For 2 weeks four chickens were given the HS diet, four the HS diet + methionine, three the RS diet and four the RS diet + methionine. Ileostomies were then performed. Quantitative measurements were made of the food intake, elimination of 'faeces' and excretion of urine. The urine was centrifuged to separate the liquid and solid fractions, and N determinations were made directly upon the liquid fraction. The solid fractions of the urine and the 'faeces' were freeze-dried, and N determinations were made upon the dry material.

*Expt 5. Intact chickens: protein and non-protein N in intestinal contents.* Twelve groups of three 6-week-old chickens were used. Three groups were given the HS diet (controls), three groups the HS diet + methionine, three groups the RS diet, and three groups the RS diet + methionine. After 24 days on these diets the chickens were

fasted for 24 h. Three groups of chickens that had been eating the HS diet were offered this diet. One group was autopsied after 2 h, the second after 6 h, and the third after 11 h. This procedure was repeated with the HS diet + methionine, the RS diet, and the RS diet + methionine, respectively. Contents from the upper and lower halves of the small intestine and caecums were collected from each chicken, frozen and freeze-dried. Proteases and total and non-protein N were determined.

*Ileostomy.* The chicken was anaesthetized by the injection of pentobarbital (Nembutal; Abbott Laboratories Ltd) into the wing vein and fastened to an operating board on its left side. The right leg extended upwards and was held in place with a rubber band. The body feathers were removed and the leg feathers were covered with gauze. An incision was made between the sternum and pelvis to permit entry into the lower part of the peritoneal cavity approximating the position of the caecums. The lower bowel was located and divided about 5–7 cm above the junction of the caecums with the colon. The caecal end was closed and returned to the abdomen. The upper end was similarly closed and fixed to the abdominal wall. A longitudinal incision was made near the closed end to serve as an enterostomy. It was attached to the abdomen with interrupted sutures and the inverted mucosa was similarly attached to the skin after the excess skin had been trimmed away.

A ring made of plastic-covered wire was sewn to the skin around the opening of the ileostomy on the chicken's right side. A plastic skirt with a slit was attached to the ring. A plastic cup which received the 'faeces' was inserted into the skirt and was held in place by a rubber band.

*Chemical methods.* The protein and the non-protein N were determined as follows. A homogenate containing about 20 mg of dry intestinal contents per ml was prepared. Total N determinations on the whole homogenate were made by the standard micro-Kjeldahl method. One vol. of 20% (w/v) trichloroacetic acid (TCA) was mixed with one vol. of homogenate; the mixture was allowed to stand for 1 h and was then centrifuged. N determinations were made upon the TCA filtrate. The protein N was calculated by subtracting the non-protein N (N of the TCA filtrate) from the total N (N of the whole homogenate). All values reported are based upon dry weight.

Proteases were determined by the method of Anson (1939). Trypsinogen in the pancreas was activated as follows: 2 ml of sample, 1·0 ml 0·1 M-phosphate buffer pH 5·6, and 2·0 ml enterokinase solution (0·5% duodenum powder; VioBin Corporation, Monticello, Ill.) were incubated for 30 min at 3°. The reaction was stopped by the addition of 5·0 ml 0·04 N-HCl. To 0·5 ml of this mixture was added 0·5 ml 0·5% (w/v) $CaCl_2$ solution; the whole was then incubated with haemoglobin substrate, and proteases were determined.

The 'trypsin' fraction of the proteases was determined as follows: 0·2 mg of a soya-bean trypsin inhibitor (Worthington Biochemical Corporation, Freehold, New Jersey) was added to the sample and the residual protease activity was determined. The difference was calculated and reported as the percentage of 'trypsin' present.

## RESULTS

### *Expt* 1. *Intact chickens*

*Body-weight.* The mean body-weights of eighteen chickens on each diet at autopsy were: HS diet 785 g, HS diet + methionine 704 g, RS diet 613 g, and RS diet + methionine 668 g.

*Pancreas.* The pancreases of the chickens given the RS diets for 6 weeks hypertrophied (Table 1). The percentage of N in the dry pancreases of the chickens after fasting was similar to that after eating in all the chickens, irrespective of the diet or the time of adaptation to it. The total N content of the pancreases of the chickens given the RS diets was markedly greater than with HS diets, since the pancreases weighed considerably more (Table 1).

The amount of the proteases in the pancreases of the fasting chickens that had been given the RS diet for 6 weeks increased markedly above the values for the chickens on the HS diets. After feeding (before adaptation), the amount of the proteases of the pancreases of the chickens given the RS diets was less than for the chickens given the HS diets. After adaptation to the RS diets for 6 weeks, the amounts of the proteases in the pancreases were higher in chickens that ate the RS diet than they were in chickens that ate the HS diets. There was little change in the percentage of the 'trypsin' fraction of the proteases during the adaptive process.

*Intestinal, caecal, and colonic contents.* The percentage of N in the contents of the upper small intestine of the chickens given RS and HS diets showed no consistent variation (Table 1); but in the lower small intestine it was always higher with the RS chickens, as it was in the caecal and colonic contents. The percentage in the caecums with both the HS and RS diets was about twice that in the contents of the lower small intestine. The percentage of N in the colonic contents was nearer that of the contents of the small intestine than that of the caecal contents.

The amount of proteases in the contents of the upper and lower halves of the small intestine of the chickens given one meal of the RS diets (unadapted) was lower than in those of the chickens given the HS diets. After adaptation there was little difference between the amount of proteases in both halves of the intestinal contents of chickens given the RS diets and HS diets.

Before adaptation, there was little difference in the amounts of proteases in the caecal contents of the chickens given HS or RS diets; after adaptation, more proteases were found in the caecal contents of the chickens given the RS diets. The amount of proteases in the colonic contents was nearer that in the contents of the lower intestine than that of the caecal contents.

The 'trypsin' fraction of the proteases of the contents of the upper half of the intestine of the unadapted chickens given the RS diets was lower than for the chickens given the HS diets; after adaptation the 'trypsin' fraction of the proteases was similar in the contents of the upper intestine of the chickens given HS or RS diets. With both the unadapted and adapted chickens the 'trypsin' fraction of the proteases in the contents of the lower half of the intestine of the chickens given the RS diets was lower than for those given the HS diets. There were similar differences in the caecal and colonic contents.

Table 1. *Expt 1. Dry weight, percentage of nitrogen, protease activity,* and percentage of trypsin in the pancreas and intestinal contents of fasting chickens and of chickens given a raw (RS) or heated (HS) soya-bean diet with or without methionine and with or without a 6-week period of adaptation to the diet*

| | Unadapted chickens | | | | | | | | Adapted chickens | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dry weight (g) | | Nitrogen content (%) | | Protease activity per g | | Trypsin activity (%) | | Dry weight (g) | | Nitrogen content (%) | | Protease activity per g | | Trypsin activity (%) | |
| Diet | Starved | Fed | Starved | Fed | Starved | Fed | Starved | Fed | Starved | Fed | Starved | Fed | Starved | Fed | Starved | Fed |
| **Pancreas** | | | | | | | | | | | | | | | | |
| HS | 0·56 (9) | 0·56 (9) | 11·6 | 11·4 | 13·5 | 5·5 | 68 | 75 | 0·56 (9) | 0·56 (9) | 11·6 | 11·4 | 13·5 | 5·5 | 68 | 75 |
| HS + methionine | | 0·56 (6) | | 11·7 | | 6·7 | | 70 | 0·57 (9) | 0·56 (9) | 12·2 | 11·8 | 11·2 | 4·6 | 68 | 75 |
| RS | | 0·51 (6) | | 11·6 | | 2·1 | | 76 | 0·82 (9) | 0·80 (9) | 12·3 | 12·0 | 26·4 | 16·4 | 67 | 69 |
| RS + methionine | | 0·52 (6) | | 11·2 | | 1·4 | | 76 | 1·02 (9) | 1·00 (9) | 12·6 | 11·9 | 19·9 | 13·0 | 68 | 69 |
| **Upper intestine** | | | | | | | | | | | | | | | | |
| HS | 0·17 | 0·38 | 9·8 | 6·9 | 0·5 | 1·8 | 69 | 78 | 0·17 | 0·38 | 9·8 | 6·9 | 0·5 | 1·8 | 69 | 78 |
| HS + methionine | | 0·31 | | 5·9 | | 1·7 | | 78 | 0·14 | 0·34 | 9·5 | 5·9 | 0·6 | 1·1 | 74 | 85 |
| RS | | 0·16 | | 6·5 | | 0·4 | | 59 | 0·15 | 0·30 | 9·5 | 5·7 | 0·5 | 0·7 | 73 | 73 |
| RS + methionine | | 0·19 | | 7·1 | | 0·4 | | 67 | 0·12 | 0·27 | 8·8 | 6·5 | 0·8 | 1·3 | 71 | 82 |
| **Lower intestine** | | | | | | | | | | | | | | | | |
| HS | 0·04 | 0·69 | 8·4 | 3·8 | 0·74 | 0·9 | 58 | 67 | 0·04 | 0·69 | 8·4 | 3·8 | 0·7 | 0·9 | 58 | 67 |
| HS + methionine | | 0·66 | | 3·2 | | 1·5 | | 72 | 0·06 | 0·54 | 7·2 | 3·8 | 0·6 | 1·2 | 72 | 71 |
| RS | | 0·36 | | 5·1 | | 0·5 | | 46 | 0·08 | 0·94 | 4·2 | 4·6 | 0·8 | 0·9 | 63 | 48 |
| RS + methionine | | 0·55 | | 5·2 | | 0·3 | | 49 | 0·04 | 0·86 | | 4·4 | 0·9 | 1·7 | 61 | 46 |
| **Caecums** | | | | | | | | | | | | | | | | |
| HS | 0·22 | 0·28 | 9·7 | 7·9 | 0·37 | 1·2 | 62 | 81 | 0·22 | 0·28 | 9·7 | 7·9 | 0·4 | 1·2 | 62 | 81 |
| HS + methionine | | 0·26 | | 8·5 | | 1·9 | | 81 | 0·27 | 0·24 | 9·6 | 8·5 | 0·2 | 1·0 | 56 | 75 |
| RS | | 0·36 | | 9·3 | | 1·6 | | 45 | 0·32 | 0·54 | 9·7 | 9·1 | 0·8 | 3·2 | 72 | 57 |
| RS + methionine | | 0·41 | | 9·5 | | 2·0 | | 40 | 0·23 | 0·40 | 9·7 | 9·5 | 0·8 | 5·3 | 54 | 48 |
| **Colon** | | | | | | | | | | | | | | | | |
| HS | 0·03 | 0·14 | | 3·8 | 0·42 | 0·7 | 50 | 76 | 0·03 | 0·14 | | 3·8 | 0·3 | 0·7 | 50 | 76 |
| HS + methionine | | 0·13 | | 3·1 | | 1·1 | | 80 | 0·03 | 0·19 | | 2·9 | 0·8 | 0·8 | 33 | 77 |
| RS | | 0·06 | | 5·5 | | 0·5 | | 46 | 0·04 | 0·13 | | 4·7 | 0·4 | 1·0 | 47 | 41 |
| RS + methionine | | 0·07 | | 5·4 | | 0·5 | | 47 | 0·03 | 0·17 | | 4·4 | 0·8 | 1·7 | 35 | 38 |

The numbers in parentheses represent the number of chickens/group. The determinations were made on pooled samples of three chickens/group.
* Expressed as m-equiv. tyrosine released in 10 min at 37°; results based on dry matter.

Table 2. *Expt 2. Chickens with ileostomies. Body-weight of chickens, and dry weight, percentage of nitrogen, protease activity,* and percentage of 'trypsin' activity of the pancreases and mixture of intestinal contents and 'faeces'† of fasting chickens and of chickens given a raw (RS) or heated (HS) soya-bean diet with or without methionine and with or without a 6-week period of adaptation to the diet*

| Diet | Body-weight (g) | Dry weight (g) | | Nitrogen content (%) | | Protease activity per g | | 'Trypsin' activity (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Starved for 23 h | Fed for 5 h | Starved for 23 h | Fed for 5 h | Starved for 23 h | Fed for 5 h | Starved for 23 h | Fed for 5 h |
| **Pancreas, unadapted chickens** | | | | | | | | | |
| HS (10) | 543 ± 108 | 0·47 ± 0·1 | 0·44 ± 0·06 | 11·2 ± 0·8 | 12·1 ± 0·7 | 4·9 ± 1·9 | 4·5 ± 1·8 | 69 ± 5 | 72 ± 7 |
| HS + methionine (5) | 535 ± 90 | — | 0·53 ± 0·2 | — | 10·5 ± 2·0 | — | 5·5 ± 1·7 | — | 67 ± 8 |
| RS (5) | 534 ± 28 | — | 0·43 ± 0·1 | — | 11·2 ± 0·4 | — | 1·5 ± 0·5 | — | 77 ± 5 |
| RS + methionine (5) | 540 ± 75 | — | 0·44 ± 0·01 | — | 11·1 ± 0·7 | — | 1·8 ± 1·0 | — | 76 ± 7 |
| **Pancreas, adapted chickens** | | | | | | | | | |
| HS (7) | 393 ± 41 | 0·43 ± 0·01 | 0·38 ± 0·05 | 12·0 ± 1·0 | 11·8 ± 0·3 | 4·1 ± 1·0 | 2·1 ± 1·7 | 76 ± 10 | 75 ± 5 |
| HS + methionine (6) | 530 ± 153 | 0·47 ± 0·01 | 0·47 ± 0·05 | 11·4 ± 0·6 | 11·4 ± 0·1 | 6·2 ± 1·6 | 2·1 ± 0·3 | 72 ± 6 | 80 ± 6 |
| RS (9) | 383 ± 61 | 0·57 ± 0·18 | 0·58 ± 0·09 | 11·8 ± 0·6 | 11·7 ± 0·2 | 11·8 ± 4·0 | 3·2 ± 1·2 | 68 ± 8 | 77 ± 3 |
| RS + methionine (8) | 434 ± 35 | 0·73 ± 0·15 | 0·71 ± 0·1 | 12·7 ± 0·2 | 12·7 ± 0·3 | 13·3 ± 1·5 | 4·9 ± 1·0 | 68 ± 6 | 75 ± 4 |
| **Intestinal contents and 'faeces', unadapted chickens** | | | | | | | | | |
| HS | — | 0·52 ± 0·6 | 1·75 ± 0·8 | 6·0 ± 2·3 | 3·9 ± 0·5 | 0·5 ± 0·1 | 0·9 ± 0·1 | 72 ± 9 | 79 ± 5 |
| HS + methionine | — | — | 2·36 ± 0·9 | — | 3·7 ± 0·5 | — | 1·0 ± 0·2 | — | 77 ± 5 |
| RS | — | — | 1·91 ± 0·7 | — | 5·4 ± 0·5 | — | 0·4 ± 0·2 | — | 44 ± 13 |
| RS + methionine | — | — | 1·91 ± 0·4 | — | 5·4 ± 0·5 | — | 0·6 ± 0·3 | — | 55 ± 6 |
| **Intestinal contents and 'faeces', adapted chickens** | | | | | | | | | |
| HS | — | 0·18 ± 0·1 | 2·04 ± 0·1 | 4·2 ± 0·5 | 3·3 ± 0·2 | 0·8 ± 0·1 | 0·9 ± 0·2 | 70 ± 1 | 75 ± 3 |
| HS + methionine | — | 0·18 ± 0·07 | 3·15 ± 0·4 | 4·4 ± 0·3 | 3·2 ± 0·5 | 0·9 ± 0·3 | 1·0 ± 0·1 | 67 ± 7 | 79 ± 4 |
| RS | — | 0·32 ± 0·1 | 2·38 ± 0·5 | 4·7 ± 0·9 | 4·3 ± 0·5 | 0·7 ± 0·02 | 1·2 ± 0·3 | 73 ± 7 | 49 ± 5 |
| RS + methionine | — | 0·34 ± 0·2 | 2·78 ± 1·2 | 4·3 ± 1·3 | 4·4 ± 0·8 | 0·9 ± 0·3 | 1·2 ± 0·3 | 61 ± 7 | 46 ± 4 |

The numbers in parentheses represent the number of chickens/group. The values are given with standard deviations.
* Expressed as m-equiv. tyrosine released in 10 min at 37°; results based on dry matter.
† The term 'faeces' is used to represent the excreta obtained through the ileostomy.

Table 3. *Expt 3. Chickens with ileostomies. Dry weight, percentage of nitrogen, protease activity,* and percentage of 'trypsin' of the pancreases and of intestinal contents and 'faeces'† of chickens starved for 18 h or 23 h and of chickens offered for 5 h a raw (RS) or heated (HS) soya-bean diet with or without methionine*

| Diet | Dry weight (g) | | | Nitrogen content (%) | | | Protease activity per g | | | 'Trypsin' activity (g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Starved for | | | Starved for | | | Starved for | | | Starved for | | |
| | 18 h | 23 h | Fed for 5 h | 18 h | 23 h | Fed for 5 h | 18 h | 23 h | Fed for 5 h | 18 h | 23 h | Fed for 5 h |
| | | | | | | Pancreas | | | | | | |
| HS | 0·47 ±0·1 | 0·48 ±0·01 | 0·47 ±0·1 | 11·1 ±0·8 | 11·4 ±0·7 | 11·2 ±0·1 | 5·0 ±1·0 | 4·9 ±0·2 | 2·8 ±0·8 | 62 ±5 | 67 ±6 | 71 ±8 |
| HS + methionine | — | — | 0·49 ±0·02 | — | — | 11·2 ±0·3 | — | — | 4·5 ±1·0 | — | — | 71 ±6 |
| RS | — | — | 0·43 ±0·02 | — | — | 10·3 ±0·1 | — | — | 1·3 ±0·3 | — | — | 77 ±4 |
| RS + methionine | — | — | 0·49 ±0·02 | — | — | 10·7 ±1·0 | — | — | 1·0 ±0·1 | — | — | 77 ±7 |
| | | | | | | Intestinal contents (IC) and 'faeces' | | | | | | |
| | | | IC | 'Faeces' | | IC | 'Faeces' | | IC | 'Faeces' | | IC | 'Faeces' |
| HS | 0·07 ±0·02‡ | 0·05 ±0·02§ | 1·06 ±0·4 | 0·74 ±0·5 | 3·3 ±0·8‡ | 5·1 ±0·9§ | 3·3 ±0·4 | 2·9 ±0·2 | 0·7 ±0·3‡ | 0·6 ±0·2§ | 1·0 ±0·1 | 0·6 ±0·2 | 65 ±5‡ | 68 ±7§ | 74 ±5 | 75 ±5 |
| HS + methionine | — | — | 0·59 ±0·05 | 1·42 ±0·6 | — | — | 4·0 ±0·7 | 3·5 ±0·1 | — | — | 1·2 ±0·2 | 0·8 ±0·1 | — | — | 76 ±2 | 79 ±6 |
| RS | — | — | 0·69 ±0·1 | 1·92 ±0·9 | — | — | 4·9 ±0·1 | 4·6 ±0·5 | — | — | 0·2 ±0·02 | 0·5 ±0·2 | — | — | 81 ±6 | 50 ±8 |
| RS + methionine | — | — | 1·04 ±0·5 | 1·45 ±0·4 | — | — | 4·6 ±0·2 | 4·7 ±0·2 | — | — | 0·3 ±0·1 | 0·8 ±0·4 | — | — | 69 ±5 | 43 ±10 |

Five birds/group. The values are given with standard deviations.
* Expressed as m-equiv. of tyrosine released in 10 min at 37°; results based on dry matter.
† The term 'faeces' is used to represent the excreta obtained through the ileostomy.
‡ Intestinal contents only.
§ Intestinal contents and 'faeces' pooled.

Table 4. *Expt 4. Intake and excretion in 'faeces'* of the nitrogenous and non-nitrogenous fractions of the diet and excretion of nitrogen in the urine of chickens given a raw (RS) or heated (HS) soya-bean diet with or without methionine*

| Diet | Intake | | Liquid fraction | Urine | | | | 'Faeces' | | | N absorbed as % of total N intake | Non-nitrogenous fraction absorbed as % of non-nitrogenous intake† |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Solid fraction | | Total N | | | | | | |
| | Food (g) | N (mg) | N (mg) | Dry wt (g) | N (mg) | mg | % of intake | Dry wt (g) | Total N (mg) | Non-nitrogenous fraction (g) | | |
| HS (4) | 47 ±10 | 1822 ±439 | 77 ±23 | 2·01 ±0·76 | 604 ±215 | 680 ±252 | 39 ±15 | 9·92 ±2·5 | 332 ±133 | 7·85 ±2·72 | 82 ±5 | 78 ±5 |
| HS + methionine (4) | 47 ±17 | 1830 ±496 | 109 ±31 | 2·02 ±0·82 | 597 ±154 | 706 ±183 | 39 ±6 | 8·08 ±0·95 | 252 ±25 | 6·51 ±0·84 | 85 ±4 | 81 ±4 |
| RS (3) | 61 ±14 | 2045 ±486 | 97 ±4 | 1·98 ±0·74 | 594 ±225 | 690 ±228 | 33 ±7 | 16·57 ±5·03 | 756 ±233 | 11·85 ±3·57 | 64 ±3 | 76 ±1 |
| RS + methionine (4) | 67 ±18 | 2312 ±643 | 137 ±32 | 2·57 ±0·60 | 764 ±170 | 901 ±215 | 40 ±10 | 16·54 ±2·65 | 736 ±140 | 11·93 ±1·81 | 68 ±2 | 77 ±4 |

The numbers in parentheses represent the number of chickens/group. The values are given with standard deviations.
* The term 'faeces' is used to represent the excreta obtained through the ileostomy.
† The values for the non-nitrogenous fraction of the food and 'faeces' were obtained by multiplying the value for N by 6·25 and subtracting this figure from the total dry weight of the food and 'faeces'.

*Expt 2. Chickens with ileostomies*

The results are shown in Table 2. The pancreases of unadapted chickens given one meal of HS diet with or without methionine did not differ significantly in protease activity from those of fasting chickens. Otherwise the results obtained in the tests on the pancreases and on the intestinal contents of chickens with ileostomies were similar to those obtained for intact chickens.

*Expt 3. Unadapted chickens with ileostomies*

The results for the pancreas (Table 3) were similar to those obtained with the unadapted chickens in previous experiments (Tables 1 and 2). Fasting levels of proteases were reached in the pancreas in 18 h, since the values were no higher after a 23 h fast.

Solids found in the intestines of the chickens after an 18 h fast were similar in amount to those found after a 23 h fast (Table 3). The percentage of N in the intestinal contents was higher after a 23 h fast than it was after an 18 h fast, but the concentration of proteases (Table 3) was similar. There was no difference in the N content of the intestinal contents and 'faeces' of the chickens given the RS diets. With chickens given the HS diets, the proportion of N was higher in the intestinal contents than in the 'faeces'. For chickens given HS diets the concentration of the proteases in the contents of the intestine was a little higher than that of the proteases in the 'faeces' (Table 3). For chickens given the RS diet the 'faeces' contained more proteases than the contents of the intestine.

In the 'faeces' little difference was seen between the concentration of the proteases with the HS and RS diets; but there was less 'trypsin' fraction with the RS diets. In the intestinal contents the concentration of the proteases was much lower with the RS diets, but the content of the 'trypsin' fraction was similar with both the HS and RS diets.

*Expt 4. Digestion of the nitrogenous and non-nitrogenous fractions of the diet*

The results are shown in Table 4. The quantity of food eaten varied widely among individual chickens: on the average, those given the RS diets ate more than those on the HS diets.

The RS diet had no consistent effect upon the excretion of N in the urine calculated as a percentage of the N intake; but more N was eliminated in the 'faeces' with RS diets than with HS diets. Consequently, less N (calculated as a percentage of the N intake) was absorbed from the intestine of chickens given the RS diet. There was no difference between the HS and RS diets in the absorption of the non-nitrogenous fraction of the diet similarly calculated.

*Expt 5. Proteases, protein and non-protein nitrogen in intestinal contents of intact chickens*

There was little difference (Table 5) in the percentage of the total N in the contents of the upper small intestine of chickens given HS or RS diets at 2 and 6 h after the

Table 5. *Expt 5. Dry weight, protease activity,* percentage of total (TN), non protein (NPN) and protein (PN) nitrogen of the contents of the upper and lower halves of the small intestine and of the caecums of groups of three intact chickens starved for 24 h and then given for 2, 6 or 11 h the diet they had previously received for 24 days*

| Diet | 2 h | | | | | 6 h | | | | | 11 h | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dry wt (g) | TN (%) | NPN (%) | PN (%) | Protease activity per g | Dry wt (g) | TN (%) | NPN (%) | PN (%) | Protease activity per g | Dry wt (g) | TN (%) | NPN (%) | PN (%) | Protease activity per g |
| **Upper intestine** | | | | | | | | | | | | | | | |
| HS | 3·61 | 5·7 | 2·9 | 2·8 | 1·00 | 3·43 | 6·5 | 3·3 | 3·2 | 0·73 | 2·35 | 5·9 | 3·2 | 2·6 | 0·46 |
| HS + methionine | 1·75 | 6·4 | 3·2 | 3·2 | 1·08 | 3·96 | 6·2 | 4·4 | 1·8 | 1·19 | 2·81 | 5·9 | 3·1 | 2·8 | 0·83 |
| RS | 1·92 | 6·8 | 2·4 | 4·4 | 0·45 | 1·69 | 6·3 | 2·7 | 3·6 | 0·38 | 2·12 | 7·2 | 3·1 | 4·1 | 0·40 |
| RS + methionine | 1·29 | 6·1 | 2·2 | 3·9 | 0·58 | 2·23 | 6·9 | 2·7 | 4·2 | 0·33 | 2·44 | 6·9 | 2·9 | 4·0 | 0·27 |
| **Lower intestine** | | | | | | | | | | | | | | | |
| HS | 5·76 | 4·0 | 2·5 | 1·5 | 0·96 | 4·19 | 4·1 | 2·5 | 1·7 | 0·83 | 3·79 | 4·0 | 2·7 | 1·3 | 1·02 |
| HS + methionine | 3·97 | 4·3 | 2·4 | 1·9 | 1·12 | 4·76 | 3·7 | 2·6 | 1·2 | 0·97 | 4·30 | 3·7 | 2·5 | 1·2 | 0·83 |
| RS | 3·93 | 6·1 | 1·7 | 4·4 | 0·22 | 8·52 | 5·3 | 1·7 | 3·6 | 0·22 | 5·16 | 5·5 | 2·1 | 3·4 | 0·13 |
| RS + methionine | 4·56 | 4·9 | 2·4 | 2·5 | 1·07 | 7·38 | 5·9 | 1·9 | 4·0 | 0·38 | 6·43 | 5·9 | 1·7 | 4·2 | 0·35 |
| **Caecums** | | | | | | | | | | | | | | | |
| HS | 2·49 | 7·1 | 1·4 | 5·7 | 0·46 | 2·82 | 6·6 | 1·4 | 5·2 | 0·37 | 2·69 | 6·5 | 1·3 | 5·2 | 0·64 |
| HS + methionine | 1·49 | 8·9 | 0·2 | 8·7 | 0·43 | 2·53 | 7·0 | 1·4 | 5·6 | 0·66 | 1·73 | 7·2 | 1·4 | 5·8 | 0·77 |
| RS | 1·56 | 10·1 | 1·4 | 8·7 | 1·72 | 3·46 | 9·4 | 1·2 | 8·2 | 1·40 | 2·24 | 9·6 | 1·0 | 8·6 | 1·92 |
| RS + methionine | 1·95 | 10·3 | 0·3 | 10·0 | 2·00 | 2·85 | 9·8 | 1·2 | 8·6 | 2·20 | 1·98 | 9·6 | 0·7 | 8·9 | 2·27 |

* Expressed as m-equiv. tyrosine released in 10 min at 37°.

diet had been offered; 11 h later, however, there was a higher percentage of N with the RS diets. There was less non-protein N, expressed as a percentage, in the contents of the upper small intestine of the chickens given the RS diets for 2 or 6 h than, correspondingly, with the HS diets; the decrease was balanced by an increase in the percentage of the protein N. After 11 h there was little difference between the chickens given RS and HS diets in the percentage of non-protein N in the contents of the upper small intestine; the higher total N content could be accounted for by an increase in the percentage of protein N.

The percentage of total N in the contents of the lower small intestine and caecums of the chickens given the RS diets was higher than with the HS diets; these higher N values were accounted for almost wholly by higher percentages of protein N.

The activity of the proteases was lower in the contents of the upper and lower halves of the small intestine of the chickens given the RS diets for 24 days than for those given the HS diets, but they were higher in the caecal contents of the chickens given the RS diets.

## DISCUSSION

The values presented in Table 1 confirm the findings of Chernick *et al.* (1948), as they do the work of others (Alumot & Nitsan, 1961; Saxena, Jensen & McGinnis, 1962), that the continuous intake of RS causes hypertrophy of the pancreas; this hypertrophy did not occur in the chickens given the HS diet. Along with this hypertrophy there was a marked increase in the proteases of the pancreas. These changes in the pancreas appear to be a response to the ingestion of the raw soya-bean trypsin inhibitor.

The findings indicate that the secretion of pancreatic juice is under some homoeostatic control which seems to respond to the level of the proteases (trypsin?) in the intestine. When the proteases in the contents of the intestine are reduced by the trypsin inhibitor of the RS, the pancreas responds with greater secretion of pancreatic juice. This may be considered as the immediate response. With continued consumption of the RS diets, the pancreas adapts by an increase in weight and in the amount of the proteases. If sufficient time for adaptation is allowed, the pancreas secretes sufficient proteases to combine with the trypsin inhibitor and, in addition, to maintain as high a level of proteases in the intestinal contents of the chickens given the RS as in those given the HS diets. Such adaptation occurred in 42 days (Table 1) but not in 24 (Table 5). This observation is in agreement with that of Alumot & Nitsan (1961).

The results suggest a feedback mechanism from the intestinal lumen to the pancreas, causing the pancreas to respond to the decrease in proteases in the contents of the intestine so as to restore the levels to normal.

Findings presented in Tables 1–5 support the conclusion of Hayward *et al.* (1936) that there is an 'unavailable essential protein fraction' that escapes digestion and utilization in chickens given RS diets. The evidence was:

(1) The percentage of N in the contents in the lower small intestine of intact chickens (Table 1) and in 'faeces' of chickens with ileostomies (Tables 2 and 3) was higher with RS diets than with HS diets. The higher percentage of N in the contents

of the lower intestine and 'faeces' of chickens given RS diets was not due to insufficient proteolytic activity, since the levels of proteases were as high there as with the HS diets (Tables 1, 2 and 3).

(2) Less N was absorbed from the intestines of chickens on RS diets than with HS diets. No such decrease was found in the absorption of the non-nitrogenous fraction of the diet (Table 4).

(3) There was more protein N in the intestinal contents of the chickens on the RS diets than with the HS diets, indicating incomplete proteolysis with the former (Table 5). This apparent incomplete proteolysis occurred in chickens adapted to RS, indicating that complete proteolysis may not necessarily be ensured by the presence of adequate proteolytic activity, as shown in this work and in the in vitro work of Riesen, Clandinin, Elvehjem & Cravens (1947), and of Liener & Fevold (1949).

Though this evidence suggests the presence in RS of an 'unavailable essential protein fraction', it does not, however, prove it, since Westfall *et al.* (1948) showed that crude preparations of soya-bean trypsin inhibitor inhibited the growth of mice on a diet containing hydrolysed casein. An 'unavailable essential protein fraction' may exist in RS; or the RS, through its trypsin inhibitor, may cause the formation in the intestinal contents of such an 'unavailable protein fraction' from the soya-bean protein, the endogenous protein, or from both.

A special situation was encountered in the upper half of the small intestine; there was little difference in the percentage of N in the intestinal contents between chickens given RS diets and chickens given the HS diets. It was probably due to the massive dilution of the dietary N with endogenous N consisting of the digestive secretions, the sloughing of mucosa (LeBlond & Walker, 1956), the passage of plasma albumin into the lumen (Campbell, Cuthbertson, Mackie, McFarlane, Phillipson & Sudsaneh, 1961) and the like. The dilution of the dietary N with the endogenous N in the duodenum of the chicken is estimated to be about tenfold (Bolton, 1961), whereas in the intestine of the dog there is said to be a fourfold dilution (Nasset & Ju, 1961), and in the rat a six- or seven-fold dilution (Nasset & Ju, 1961). Other work indicates a lesser dilution in the rat (Twombly & Meyer, 1961). This secretion of endogenous N into the upper small intestine may obscure processes going on there, one of which is digestion (Pisano, Paine & Taylor, 1959).

Experiments with germ-free and conventional chickens show no recognizable microbial activity in the intestinal contents, but they do show active microbial activity in the caecal contents. The percentage of the N in the caecal contents of conventional chickens is about twice as high as in the caecal contents of germ-free chickens (Lepkovsky *et al.* 1964). Our results with intact chickens (Table 1) also indicate an abundance of microbial activity in the caecums. The percentage of N in the caecal contents was about twice that in the contents of the lower intestine, indicating that the caecums represent a cul-de-sac with their own environment. The contents of the colon are little affected by the contents of the caecums which are expelled periodically in large amounts and mix minimally with those of the colon. The high level of proteases of the caecal contents, especially of chickens adapted to RS diets (Table 1), also suggests microbial activity. Interpretation is difficult, however, since it is not possible

to differentiate between proteases originating in the pancreas and those synthesized by intestinal bacteria, and the methods for determining proteases are not specific. The use of chickens with ileostomies made possible the collection of intestinal contents that were not contaminated with caecal contents and therefore were not affected by microbial activity originating in the caecums.

The use of chickens with ileostomies made possible the fractionation of the pancreatic juice in response to the meal. In intact chickens the first pancreatic juice entering the intestine was largely lost with the excretion of faeces. In chickens with ileostomies the first secretion of pancreatic juice was collected with the 'faeces'. The later secretion was collected with the intestinal contents. The quantities of proteases collected with the 'faeces' of the unadapted chickens differed little with the HS and RS diets, as though the trypsin inhibitor of the RS had failed to act upon the trypsin of the pancreatic juice (Table 3). However, the trypsin inhibitor did act upon the trypsin, since the 'trypsin' fraction of the proteases was reduced. Presumably the decrease in the 'trypsin' fraction was balanced by an increase in the other pancreatic proteases. These measurements would have been falsely interpreted if the 'trypsin' fraction of the proteases had not been determined. On the other hand, the content of proteases collected with the intestinal contents of chickens given the RS diets was much less than with the HS diet. This decrease would usually be interpreted as establishing the action of the trypsin inhibitor of the RS. However, the 'trypsin' fraction of the proteases of the intestinal contents of the chickens on the RS diet was as high as with the HS diet.

One explanation of these results may be offered. The 'faeces' contained the first pancreatic juice that was secreted into the intestine when the RS diet was first eaten. Part of the trypsin was inactivated by the trypsin inhibitor of the RS. This accounts for the lower 'trypsin' fraction in the 'faeces' of the chickens fed on the RS diet. The pancreatic juice collected with the intestinal contents represented a later secretion of pancreatic juice and the 'trypsin' fraction was similar with HS or RS diets. Either the pancreatic juice contained a sufficient excess of trypsin to replace that inactivated by the trypsin inhibitor, or there was no dietary trypsin inhibitor in the intestinal contents. The values in Table 3 indicate that the trypsin inhibitor was present in the intestinal contents, since a higher percentage of N was found with RS diets than with HS diets. If these considerations reflect the nature of the processes going on in the pancreas, the proportion of the trypsin in the pancreatic juice should increase in the 5 h period during which the RS diet was given. This could be proved by cannulating the pancreatic duct, leading the pancreatic juice to the outside and returning it to the pancreatic duct or to the duodenum. This preparation would enable the investigator to sample the pancreatic juice as it flowed through the tube and to measure the composition and rate of flow of the pancreatic juice. Our persistent attempts for over 10 years to make such a preparation have resulted in failure.

A measure of the magnitude of the secretory activity of the pancreas is shown in Table 6. Much more proteases were secreted by the pancreas in 24 h than were found in the pancreas at fasting when they were there at their highest; at times the amount collected in 24 h with the 'faeces' of chickens given the HS diets was two or more

times the amount in the pancreas at fasting. These estimates are minimal, since no data are available to indicate how much, if any, of the proteases that enter the intestine of the chicken are inactivated as they are carried by the intestinal contents until they are passed out through the ileostomy with the 'faeces'. As much proteases were collected with the 'faeces' of chickens given the RS diet for 24 h as were found in the pancreases at fasting, in spite of the losses of trypsin through the action of the raw soya-bean trypsin inhibitor.

Table 6. *Protease activity* of the pancreases of chickens at fasting compared with the total protease activity* of the 'faeces' and intestinal contents collected from chickens given food for 5 or 24 h*

| Diet | Pancreas | 'Faeces' + intestinal contents | |
|---|---|---|---|
| | Starved for 23 h | Fed for 5 h | Fed for 24 h |
| | Unadapted chickens | | |
| HS | 2·3 ± 1·0 (4) | 1·5 ± 0·45 (5) | 5·2 ± 2·5 (27) |
| HS + methionine | — | 2·5 ± 1·4 (5) | — |
| RS | — | 0·8 ± 0·3 (4) | — |
| RS + methionine | — | 1·2 ± 0·4 (6) | — |
| | Adapted chickens | | |
| HS | 1·8 ± 0·4 (3) | 1·9 ± 0·4 (4) | 5·1 ± 1·5 (7) |
| HS + methionine | 3·1 ± 1·0 (3) | 3·3 ± 0·8 (3) | 4·4 ± 1·6 (6) |
| RS | 7·1 ± 3·9 (3) | 2·7 ± 0·7 (6) | 8·4 ± 0·7 (9) |
| RS + methionine | 9·9 ± 2·9 (3) | 3·6 ± 1·0 (5) | 8·5 ± 0·5 (8) |

The numbers in parentheses represent the number of chickens/group. For the 24 h group there were two 24 h collections from each chicken.

* Expressed as m-equiv. tyrosine released in 10 min at 37°; results based on dry matter.

## SUMMARY

1. Raw and heated soya beans were given to intact chickens and to chickens with ileostomies.

2. The pancreas responded to raw soya beans by (*a*) immediately increasing the secretion of pancreatic juice; and (*b*) hypertrophy accompanied by an increase in the concentration of the proteases in the pancreas. This adaptive response continued for at least 6 weeks or longer.

3. The proteases were at their highest in the pancreases of fasting chickens. After eating the heated soya-bean diets there was almost always a decrease in the proteases; the decreases were much greater with raw soya beans. There was little change in the percentage of nitrogen in the pancreas after eating, whether before or after adaptation to the raw soya-bean diets.

4. The responses in the pancreases were reflected in the intestinal contents thus: (*a*) After the initial ingestion of raw soya beans there was a decrease in the proteases of the contents of the small intestine and colon of intact chickens but not in those of the caecums. (*b*) The proteases in the contents of the small intestines of chickens that had eaten the raw soya-bean diets for 6 weeks were as high as for chickens on the heated soya-bean diet. The proteases of the caecal contents were higher in chickens given the

raw soya-bean diet than in those given the heated soya-bean diet. (*c*) Three weeks was insufficient time for these adaptive responses.

5. An 'unavailable protein fraction' was found in the intestinal contents as indicated by: (*a*) There was a greater percentage of N in the contents of the lower half of the small intestine of chickens given raw soya-bean diets as compared with those given the heated soya-bean diets. This increase was not seen in the upper half of the small intestine. It was probably masked by the massive dilution of the dietary N by endogenous N. (*b*) There was a decrease in the absorption of N from the small intestine of the chickens given raw soya-bean diets. (*c*) A larger amount of protein N was found in the contents of the small intestine of the chickens given raw soya-bean diets than in those given the heated soya-bean diets.

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