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Effect of Raw Legume Diets on Disaccharidase Activity in the Small Intestine of Chicks

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The effect of four raw legume diets: field beans *(Vicia faba)* (RFB), navy beans *(Phaseolus vulgaris)* (RNB), soybeans *(Glycine soja)* (RSB) and bitter vetch *(Vicia ervilia)* (RBV), on disaccharidase activities in chick small intestine have been studied. Maltase and sucrase activities, which vary with age, were determined in 1 to 60 day old animals. RFB and RBV diets had no effect on maltase activity and only increased sucrase activity in 60 day old chicks. Both maltase and sucrase activities decreased in chicks on RSB diet, regardless of their age, and the decrease was even more pronounced in chicks on RNB diet. Contrarywise, chicks fed on autoclaved navy beans and soybeans showed a considerably higher activity of these disaccharidases.

It is known that many legumes in the raw state, when used as the main protein source in experimental diets, induce physiologic alterations and support low growth rates (1, 4, 14). POPE *et al.* (18) reported that the capacity of the small intestine to accumulate methionine actively decreased in rats fed on raw soybeans. JAFFÉ (8) showed a reduction in glucose absorption in rats fed on raw navy beans. Published data from our laboratory have likewise shown sugar absorption inhibition in chicks fed on different raw legumes (13). On the other hand, intestinal disaccharidases are located in the brush border membrane and are believed to be important in sugar absorption (3, 5). Since the available information on the effects of raw legumes on disaccharidase enzymes is so scant, we undertook this project to study the maltase and sucrase activities in the small intestine of chicks fed on one of the following four raw legumes: field beans *(Vicia faba)* (RFB), navy beans *(Phaseolus vulgaris)* (RNB), soybeans *(Glycine soja)* (RSB) and bitter vetch *(Vicia ervilia)* (RBV).

Materials and Methods

One-day-old White Leghorn chicks were placed and kept in battery brooders with

raised wire floors. In one set of experiments they were divided into five equal groups of 24 chicks each. The control group was fed a commercial diet, whereas the remaining four groups had a raw legume as their main protein source. Table I presents the composition of these diets. On days 15, 21, 30 and 60 six animals from each group were weighed and sacrificed by decapitation. In another set of experiments one group of 14 chicks was fed a diet of autoclaved navy beans (ANB) and a second group a diet of autoclaved soybeans (ASB). The autoclaving treatment of navy beans and soybeans was carried out by heating the meal at 15 psi for 20 min. On days 15 and 30 seven animals from each group were sacrificed after a 12 h fast. In all the experiments food and water were given *ad libitum*.

Tissue preparation. The intestines were removed immediately after killing. Then three segments of approximately equal size were obtained from the small intestine and their possible variations in enzyme activity were determined.

The first segment was taken from the gizzard end, the second included the yolk stalk, and the third terminated in the ileocaecal junction. Since the second segment showed the highest enzyme activity, only this one was used for further analyses. A small specimen from this same segment was taken for histological examination. The intestinal segment was washed with ice-cold 0.9 % NaCl and opened longitudinally. The mucosa was scraped off with a glass microscope slide and homogenized in a Potter-Elvehjem homogenizer in distilled water. The volume of the homogenate was centrifuged at 800 *g* for 10 min at 4° C in a SORVALL centrifuge. The supernatant was used in the estimation of enzyme activities.

The intestinal segments for histology were fixed in formaldehyde and embedded in paraffin.

Disaccharidase assays. Maltase and sucrase activity was determined by the DAHLQUIST method (6), modified by SIDDONS (20), which measures the produced glucose after incubation of substrate and supernatant of homogenate. Incubation was carried out during 30 min at 37° C. One unit of disaccharidase activity is defined as the amount that hydrolyses 1 μmole of disaccharide in 30 min at 37° C.

The protein content of the homogenates was assayed by the method of LOWRY *et al.* (15). Bovine plasma albumin was used to prepare a standard curve.

The data were statistically evaluated by Student's t test.

Results

The effect of different legume diets on the activity of some disaccharidases in chicken small intestine are shown in tables II and III.

Maltase activity increased with the age, especially up to the 30th day. This finding is in agreement with the results obtained by SIDDONS (20). Maltase activity was significantly reduced in chicks fed RNB diets at all the studied ages (table II). This reduction was particularly accused by birds on RNB diet and ranged from 66 % to 59 % at the four studied ages. Chicks fed on RBV and RFB did not show any significant differences.

Similar results were obtained for sucrase activity (table III). Chicks fed RNB and RSB diets showed a reduction in sucrase activity. The greatest decrease was observed with RNB diet. Sucrase activity increased in 56-day-old chicks fed RBV and RFB diets, but no significant variations were observed in younger animals.

The results obtained when chicks were fed with the autoclaved legumes are shown in table IV. The data indicate that the heat treatment increased both sucrase and maltase activities.

Light microscopic examinations did not show any significant abnormalities in the mucosa of animals fed on legumes.

Table I. *Composition (%) of experimental diets.*

	RFB	RSB or ASB	RNB or ANB	RBV
Raw field beans meal	50	—	—	—
Raw or Autoclaved soybeans meal	—	50	—	—
Raw or Autoclaved navy beans meal	—	—	30**	—
Raw bitter vetch meal	—	—	—	30**
Maize meal	35	38	46	46
Fish meal	11	5	11	11
Barley meal	2	4	11	11
$CaCO_3$	1	1	1	1
$CaHPO_4$	0.5	0.5	0.5	0.5
NaCl	0.30	0.30	0.30	0.30
Supplement*	0.20	0.20	0.20	0.20
Crude protein (N × 6.25) (%)	21.1	22.3	22.7	22.7

* The supplement for 1 kg diet contained: Vitamin A, 5,250 IU; Vitamin D_3, 520 UI; Riboflavina, 4 mg; Nicotinic acid, 20 mg; Cu, 3 mg; Fe, 35 mg; Mg, 300 mg; Mn, 50 mg.

** Navy beans and bitter vetch could not be used at 50 % since at this proportion they produced severe diarrheas and in some cases death.

Table II. *Intestinal maltase activity in chicks fed diets containing different legumes.* Each value represents the mean of duplicate determination of 6 chicks ± S.E.M.

Diet	Maltase activity	Days on diet			
		15	21	30	60
Control	U/mg prot.	28.06±1.23	32.01±1.05	37.25±1.38	37.67±0.38
	U/cm int.	24.14±1.03	31.05±1.08	37.96±1.74	37.71±1.25
RFB	U/mg prot.	28.53±1.33	31.85±1.40	38.26±0.67	39.31±2.94
	U/cm int.	24.59±1.52	30.40±2.90	36.50±3.27	35.90±2.62
RVB	U/mg prot.	26.17±0.34	34.52±0.73	35.85±2.40	43.71±2.79
	U/cm int.	23.45±1.22	31.08±2.16	35.49±1.61	42.03±3.25
RSB	U/mg prot.	22.37±0.54*	24.73±0.90*	31.57±1.06*	32.44±0.65*
	U/cm int.	19.73±0.67*	25.43±0.85*	30.50±1.32*	31.83±1.27*
RNB	U/mg prot.	9.48±0.36*	9.10±0.42*	9.83±0.83*	15.59±0.99*
	U/cm int.	9.78±1.01*	10.21±2.30*	11.39±1.02*	16.01±1.90*

* Differs significantly from control value for the same days of the experiments $P < 0.001$.

Table III. *Intestinal sucrase activity in chicks fed diets containing different legumes.* Each value represents the mean of duplicate determination of 6 chicks ± S.E.M.

Diet	Sucrase activity	Days on diet			
		15	21	30	60
Control	U/mg prot.	1.87±0.10	2.58±0.20	3.18±0.16	3.36±0.14
	U/cm int.	1.50±0.13	2.97±0.17	3.49±0.13	3.70±0.12
RFB	U/mg prot.	1.94±0.15	2.63±0.22	3.48±0.04	4.66±0.36*
	U/cm int.	2.05±0.20	3.15±0.20	3.89±0.12	5.53±0.25*
RVB	U/mg prot.	2.02±0.14	3.01±0.13	3.12±0.17	4.61±0.43*
	U/cm int.	2.10±0.15	3.08±0.21	3.06±0.21	5.36±1.02*
RSB	U/mg prot.	1.36±0.08*	1.82±0.16*	2.53±0.05*	2.58±0.11*
	U/cm int.	1.35±0.15*	1.80±0.19*	2.59±0.15*	2.60±0.18*
RNB	U/mg prot.	0.70±0.03*	0.84±0.04*	1.20±0.11*	1.63±0.15*
	U/cm int.	0.61±0.04*	0.84±0.08*	1.44±0.02*	1.80±0.17*

* Differs significantly from control value for the same days of the experiments $P < 0.001$.

Table IV. *Intestinal maltase and sucrase activities in chicks fed on autoclaved navy beans (ANB) and soybeans (ASB).* Significant differences from the activity of chicks fed on RNB and RSB diets. * $p < 0.001$; ** $p < 0.01$.

Diet	Days on diet	Maltase activity U/mg prot.	Sucrase activity U/mg prot.
ANB	15	19.20 ± 0.48*	1.40 ± 0.11*
	30	30.40 ± 0.81*	2.82 ± 0.05*
ASB	15	24.39 ± 0.34**	1.77 ± 0.06*
	30	34.07 ± 0.65**	2.90 ± 0.10**

Discussion

It has been shown that diet can effect disaccharidase activity in different animals (7, 17, 21). In this study RFB and RBV diets did not effect disaccharidase activity until the 60th day when an increase in sucrase activity was observed. Maltase and sucrase activities, however, decreased in chicks given RSB diet; the decrease was even more notable with the RNB diet.

In rat and man, induction of sucrase and maltase activities by specific disaccharides has been reported (2, 19). The impaired digestion of carbohydrates due to the amylase inhibitors of RNB and RSB (9, 16), could result in a low formation of disaccharides, which would lead to the assumption that the reduction of maltase and sucrase ativities in RNB and RSB fed chicks was due to the deficiency of specific disaccharides in the gut. However, SIDDONS (21) has reported that the induction of disaccharidases in the chick depends on carbohydrates but is independent of the carbohydrate form. It does not seem, therefore, that the reduction in maltase and sucrase activity from RNB and RSB diets can be explained by this mechanism. These results can be more readily explained by the action of a possible thermolabile factor in these legumes on intestinal epithelial cells. Alterations in intestinal mucosa of quail caused by hemagglutinin have been suggested by JAYNE-WILLIAMS and HEWIT (10) as well as by JAFFÉ (8) who found that a navy beens diet produced an intestinal absorption decrease in rat. This author postulated that the absorption deficiency was due to the association of hemagglutinins with the membrane of intestinal epithelial cells. If hemagglutinins are the thermolabile factor responsible for the inhibition reported here, they would account for the greater inhibitory effect of RNB as compared to that of RSB, notwithstanding the smaller proportion of legume in the former. RNB hemagglutinins have a remarkable resistance to gastric pepsin action whereas RSB ones can be partly digested by this enzyme (8).

The light microscope examination did not show any significant changes in the mucosa of chicks fed on raw legume diets. This lack of correlation between enzyme variations and morphological changes has also been reported in man and rat. THUS, KUMAR and CHASE (12) showed variation in lactase and peptidase activities in undernourished rats whose mucosa appeared to be normal under light microscope examination, KNUDSEN *et al.* (11) observed a decrease in the activities of sucrase, maltase and lactase during periods of total caloric deprivation of patients, while no histologic changes were observed.

Resumen

Se estudia el efecto de la ingestión de dietas cuya principal fuente proteica es una leguminosa cruda: habas *(Vicia faba)*, judías *(Phaseolus vulgaris)*, soja *(Glycine soja)* y yeros *(Vicia ervilia)* sobre la actividad de disacaridasas en intestino delgado de pollo. La actividad de los enzimas se determina en edades comprendidas entre 1 y 60 días. Las dietas que contienen habas o yeros no producen efecto sobre la actividad de la matalsa y sacarasa exceptuando un aumento en la actividad de sacarasa a los 60 días de edad. Las dietas que contienen

soja o judías originan una disminución en la actividad de estos enzimas, que se recupera en parte por tratamiento térmico en autoclave de las leguminosas.

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