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Effect of Raw Legume Diets on Intestinal Absorption of D-Galactose by Chick

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The effect of four raw legume diets on the intestinal absorption of D-galactose and oxygen consumption were studied in chick. Field beans *(Vicia faba)*, soybeans *(Glycine soja)*, bitter vetch *(Vicia ervilia)*, and navy beans *(Phaseolus vulgaris)*, were used. The intestinal absorption was determined by both *in vivo* and *in vitro* techniques.

In vivo, only navy beans and soybeans inhibit intestinal transport of D-galactose, while *in vitro* all the diets do. Oxygen consumption by intestinal rings increases in chicks fed on bitter vetch diet.

Ingestion of raw legumes produces varied physiological effects, usually accompanied by growth inhibition in animals. Such effects have been attributed to factors proper to these legumes, although their exact mechanism of action is still unknown (3, 8, 9). Due to the scant information available at present on the influence of legumes on intestinal absorption, and to the fact that some of these legumes are nutritionally important for both humans and animals, this study on the effect of diets containing raw field beans (FB) *(Vicia faba)*, raw soybeans (SB) *(Glycine soja)*, raw bitter vetch (BV) *(Vicia ervilia)*, and raw navy beans (NB) *(Phaseolus vulgaris)*, on the absorption of D-galactose by the chick small intestine has been done.

Materials and Methods

White Leghorn male chicks, 1 day old, were divided into five equal groups and housed in battery brooders with raised wire floors. The control group was fed a standard diet. The remaining four groups were fed raw legume diets as the main protein source. The composition of the diets is given in table I.

Absorption experiments were conducted on 15-35 day old chicks, subjected to 24 h fast prior to the experiment and anaesthetized with 12.5 % urethane. *In vivo* experiments were carried out with the SOLS and PONZ technique (13), described in detail for chicks by RUANO *et al.* (12). *In vitro* experiments were made with the CRANE and MANDELSTAM tech-

nique (2). The intestinal rings were incubated in KREBS-HENSELEIT (7) bicarbonate buffer in a thermostatic bath at 39° C and were bubbled with carbogen (95 % O_2 and 5 % CO_2) throughout the entire incubation period. Sugar was determined by the SOMOGYI (14) method. Oxygen consumption was measured by the Warburg direct method in an oxygen atmosphere at 80 oscillations/min and 3 cm amplitude in each oscillation. In these experiments Krebs-Henseleit phosphate buffer was used.

Results and Discussion

Table II shows the results from the *in vivo* absorption experiments. Four successive absorption periods of 10 minute duration were carried out on each chick. The amount of galactose absorbed in each animal during the four periods remained constant in all the experimental groups; it was significantly inhibited in the groups fed on SB (43 %) and NB (50 %) diets.

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

	FB	SB	NB	BV
FB meal	50	—	—	—
SB meal	—	50	—	—
NB meal	—	—	30**	—
BV 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	0.3	0.3	0.3	0.3
Supplement	0.2	0.2	0.2	0.2
Crude protein (N × 6.25) (%)	21.1	22.3	22.7	20.7

* The supplement for 1 kg diet contained Vitamin A: 5250 IU; Vitamin D_3: 520 IU; Riboflavin: 4 mg; Nicotinic acid: 20 mg; Cu: 3 mg; Fe: 35 mg; Mg: 300 mg; Mm: 50 mg.

** NB and BV could not be used at 50 % since at this proportion they produced severe diarrheas and in some cases death.

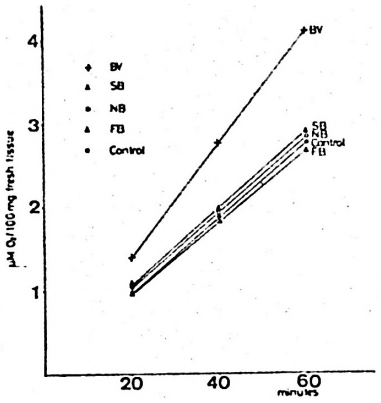

Fig. 1. *Time-course oxygen uptake by intestinal rings of chicks fed on different legume diets.*

These results are comparable to those obtained in rat by JAFFÉ (4) and JAFFÉ *et al.* (5) with navy beans, and BELLO *et al.* (1) with soybeans. D-galactose is actively transported by the intestine. The intestinal oxygen uptake of chicks fed on SB and NB diets (fig. 1) did not decrease and thus the inhibition of galactose absorption in these chicks could hardly be a result of an interference of these legumes with the energetic process. It could be due the effect of substances present in these legumes which might either alter the enterocyte membrane permeability or decrease the affinity of the carrier system for the sugar. POPE *et al.* (11) similarly observed that the absorption of an amino acid actively transported was inhibited in rats fed on raw soyben. KAKADE and EVANS (6) reached similar results with rats fed on navy beans and considered the action on intestinal cells of hemaglutinines contained in this legume as a possible cause.

Table III gives the amounts of D-galactose accumulated by intestinal rings of chiks fed on these experimental diets. The final concentration of galactose in the intestinal tissue showed an inhibition of 43 % (NB), 20 % (FB), 23 % (BV) and

Table II. *Effect of raw legume diets on the intestinal absorption of galactose* in vivo. Absorption period, 10 min. Galactose concentration 2.77 mM. The results are expresed in μmol sugar absorbed/cm intestine, and are accompanied ± SEM. In each animal four successive absorption periods were studied. Number of animals in brackets.

Diet	Absorption (μmol galactose/cm intestine)				Inhibition %
	1st	2nd	3rd	4th	
Control	0.28±0.005	0.29±0.005	0.28±0.004	0.28±0.004 (20)	
FB	0.30±0.010	0.29±0.010	0.29±0.011	0.29±0.009 (18)	n.s.
BV	0.24±0.012	0.25±0.012	0.24±0.012	0.25±0.013 (18)	n.s.
SB	0.16±0.010	0.16±0.009	0.15±0.011	0.15±0.010 (19)	43
NB	0.13±0.012	0.14±0.011	0.13±0.009	0.14±0.010 (14)	50

38 % (SB). It was observed that legumes FB and BV originated a decrease in absorption in *in vitro* experiments but not in *in vivo* experiments. The differences observed in the results between *in vivo* and *in vitro* techniques, coul be explained by the fact that in *in vivo* condition the experimental solution did not circulate in the intestinal loop. The absence of circulation brings about a rapid fall in the concentration of sugar, which masks the minor inhibition (10).

Figure 1 shows oxygen uptake by intestinal rings of chicks fed on the above legume diets. Oxygen uptake increases in animals fed on BV diet. This striking result might be due to thinner intestine in these animals than those in the other groups. If such is the case, the mucosal layer, with higher metabolic activity, would have more quantitative importance in oxygen consumption than the other layers of the intestine. However, further experiments with just the mucosal layer are necessary to check this hypothesis.

Table III. *Effect of raw legume diets on the galactose accumulation by intestinal rings of chicks.*

15-20 day-old animals were used. Incubation for 10 min in 5 ml of medium containing 2 mM galactose. The results are expressed as a final concentration of galactose in tissue, $[mM]_F$, and as a μmol sugar transported/100 mg tissue, and are accompanied ± SEM.

Diet	$[mM]_F$ tissue	μmol galactose/100 mg	Inhibition %
Control	8.51±0.21	0.68±0.02	
FB	6.25±0.18	0.50±0.03	20
BV	6.62±0.15	0.53±0.02	23
SB	5.27±0.14	0.42±0.02	38
NB	4.87±0.16	0.39±0.02	43

Resumen

Se ha estudiado el efecto de dietas cuya principal fuente proteica es una leguminosa, habas *(Vicia faba)*, soja *(Glycine soja)*, yeros *(Vicia esvilia)* y judias *(Phaseolus vulgaris)*, sobre la absorción intestinal *in vivo* e *in vitro* de D-galactosa en pollo.

Las dietas con soja o judías producen inhibición en la absorción intestinal de D-galactosa *in vivo* e *in vitro*, mientras que si contienen habas o yeros únicamente producen inhibición en los experimentos *in vitro*. El consumo de oxígeno por anillos intestinales aumenta únicamente en los pollos alimentados con yeros.

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