INTESTINAL ABSORPTION OF D-GLUCOSE, D-GALACTOSE AND L-LEUCINE IN MALE GROWING RATS FED A RAW FIELD BEAN (Vicia faba L.) DIET \(^1,2,3\)

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Summary

Male growing rats were fed diets containing either raw field bean (Vicia faba L.) or casein as the only source of protein. Diets were fed ad libitum for 5 weeks. Body weight gain was significantly impaired, and rates of both in vivo and in vitro intestinal absorption of D-glucose, D-galactose and L-leucine were reduced, in rats fed the legume diet. The addition of methionine to the bean diet did not alter either growth or intestinal absorption rates. No significant differences in intestinal oxygen uptake were found between the treatment groups. It is suggested that the antinutritive substances contained in the raw legume Vicia faba are responsible for the inhibitory effects observed in this experiment.

(Key Words: Antinutritive Substances in Legumes, Intestinal Absorption, Field Bean [Vicia faba L.], D-Glucose, D-Galactose, L-Leucine.)

Introduction

Although legumes are widely used as protein sources in human and animal nutrition, it has long been known that ingestion of these plants in the raw state produces various physiological effects, usually accompanied by inhibition of growth. Previous studies in our laboratory (Bello et al., 1972; Santidrian, 1981) and others (Boulter et al., 1976; Jamalian et al., 1976) have shown that growing rats and chickens fed diets containing the raw legume field bean (Vicia faba L.) as the major source of protein suffer a significant reduction in growth rate. The depression of growth is associated with an inefficient utilization of the dietary nutrients and an increase in urinary output of total N, urea and creatinine. Moreover, we have observed increases in the activity of several enzymes involved in the catabolism of amino acids in both rats and chickens (Cenarruzabeitia et al., 1979). This antinutritive effect of the field bean has been attributed to the presence of a number of factors in the bean, such as trypsin inhibitors, globulins, hemagglutinins, saponins and tannins (Warsy and Stein, 1973; Davidson, 1977; Marquardt et al., 1975, 1976, 1977; Eggum, 1980; Liener, 1980), and to a deficiency of sulfur-amino acids in the protein of the legume (Boulter et al., 1976).

Despite the importance of the nutritional value of the legume Vicia faba, there is little published information on the effects of these seeds on the intestinal absorption of sugars and amino acids. In a recent paper (Santidrian et al., 1981), we reported that feeding growing chickens diets containing different raw legumes as the major source of protein caused a significant reduction in the rates of in vivo intestinal absorption of both D-galactose and L-leucine. This paper reports results on the in vivo and in vitro intestinal absorption of D-glucose, D-
galactose and L-leucine in growing male rats fed a diet containing field beans as the sole source of protein, with or without added methionine. The rate of oxygen uptake by intestinal rings is also reported. Results are compared with those obtained for growing male rats fed a casein control diet.

**Materials and Methods**

Twenty Wistar male rats, 40 days old and weighing 100 to 110 g, were randomly assigned to two groups of 10 each. The animals were housed in cages with wire floors in a room maintained at 23 °C. The rats were fed for 5 weeks diets containing either casein or raw field bean (*Vicia faba* L.) as the sole source of protein. Another 20 rats were fed the same casein and field bean diets, but with methionine added at .6 g/100 g diet. The diets were formulated according to AOAC (1970) recommendations (PER procedure), and the compositions are given in table 1. Both feed and water were supplied *ad libitum*. The total protein content of each diet (N x 6.25) was 12%. Body weight changes were recorded every day for all rats.

*In vivo* and *in vitro* intestinal absorption trials were carried out at the end of the 5-week feeding period. *In vivo* experiments were conducted according to the technique of Sols (1956). Rats were fasted for 24 hr before the start of the experiment. The animals were kept in a glass cabinet (95 x 60 x 75 cm) in which the temperature was maintained between 35 and 37 °C. They were anesthetized with 12.5% urethane and placed on their backs on an appropriate restrainer holder. An abdominal incision of 2.5 to 3 cm was made, and two catheters were inserted into the upper part of the small intestine. The animals were infused with the ingoing catheter, and the portion of the infusate that was not absorbed was collected with the outcoming catheter. For each animal, five successive 20-min absorption periods were studied. Concentrations of 2 mM of D-glucose and D-galactose and 20 mM of L-[14C (U)]-leucine were assayed.

*In vitro* intestinal absorption experiments were performed by the method of Crane and Mendelstam (1960). Intestinal rings were incubated in Krebs-Henseleit bicarbonate buffer in a thermostatic bath at 39 °C and were bubbled with carbogen (95% O2 and 5% CO2) throughout the incubation period (10 min). Sugars were determined by the method described by Somogy (1952). Liquid scintillation counting (Packard model 2002) was used to determine leucine concentrations. Oxygen consumption was measured by the Warburg direct method in an oxygen atmosphere at 80 oscillations/min, with 3 cm amplitude of each oscillation (Lasheras et al., 1980). Statistical evaluations were carried out by analysis of variance. For body weight gain, *in vitro* intestinal absorption of D-galactose and L-leucine and intestinal O2 uptake, two-way (diet and methionine) analysis of variance was used, and for *in vivo* intestinal absorption of D-glucose, D-galactose and L-leucine, three-way (diet, methionine and periods) analysis of variance was used. Least Significant Differences were computed to identify significant differences between treatments (Snedecor, 1956).

**Results and Discussion**

Results are summarized in tables 2 and 3. Compared with the casein-fed animals, the rats fed the raw-legume diet displayed a reduction (P<.01) in rate of growth. The results agree with our previous observations (Bello et al., 1972; Santidrian 1981; Santidrian et al., 1981) and those of Ward et al. (1977). Growth rate was not significantly altered by the addition of methionine to either the casein or the raw-legume diet. The impairment in growth rate caused by the raw field bean was not due to a reduction in feed intake, nor can it be attributed to an amino acid deficiency in the protein of the legume, because previous studies have shown that casein and *Vicia faba* seed proteins have similar amino acid profiles, with both slightly deficient in sulfur-amino acid (Bello et al., 1972; Boulter et al., 1976). Furthermore, this study confirms that supplementation with methionine does not improve the nutritional value of the raw legume.

In the *in vivo* intestinal absorption experiments, animals fed the raw legume diet exhibited reductions (P<.01) in the rates of absorption of D-glucose, D-galactose and L-leucine in each of the five periods studied. Moreover, this inhibi-
TABLE 1. COMPOSITION OF DIETS (PERCENTAGE)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Casein 12%</th>
<th>Casein 12%+ methionine</th>
<th>Vicia faba</th>
<th>Vicia faba+ methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>13.2</td>
<td>13.2</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Methionine</td>
<td>...</td>
<td>.6</td>
<td>...</td>
<td>.6</td>
</tr>
<tr>
<td>Raw V. faba(^a)</td>
<td>...</td>
<td>...</td>
<td>53.0</td>
<td>53.0</td>
</tr>
<tr>
<td>Potato starch</td>
<td>67.3</td>
<td>66.7</td>
<td>33.0</td>
<td>32.4</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>5.0</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>4.9</td>
<td>4.9</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Mineral mix(^b)</td>
<td>4.6</td>
<td>4.6</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Vitamin mix(^c)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Total protein</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

\(^a\) *Vicia faba* composition (percentage): protein, 22.6; ether extract, 2.3; ash, 2.8; and, crude fiber, 6.9.

\(^b\) Mineral mix (percentage): NaCl, 13.92; KI, .079; KH\(_2\)PO\(_4\), 38.91; MgSO\(_4\)\(7\)H\(_2\)O, 5.73; CaCO\(_3\), 38.14; FeSO\(_4\)\(7\)H\(_2\)O, 2.7; MnSO\(_4\)\(4\)H\(_2\)O, 4; ZnSO\(_4\)\(4\)H\(_2\)O, .055; CuSO\(_4\)\(5\)H\(_2\)O, .048; CoCl\(_2\)\(6\)H\(_2\)O, .002.

\(^c\) Vitamin mix contained (in milligrams/gram): vitamin K\(_1\), .15; choline, 200; niacin, 4; calcium pantothenate, 4; riboflavin, .8; thiamin hydrochloride, .5; pyridoxine hydrochloride, .5; folic acid, .2; biotin, .04; and (in IU): vitamin A, 2,000; vitamin D, 200; vitamin E, 10.

The results of this study suggest that the reduction in the intestinal absorption of D-glucose, D-galactose and L-leucine both in vivo and in vitro is responsible, at least in part, for the growth inhibition exhibited by rats fed raw field bean. We suggest that this fact be taken into account when field bean diets are used in animal nutrition.
<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weight gain, g/day ± SEM</th>
<th>D-glucose, 2mM/cm intestine/5 min</th>
<th>D-galactose, 2mM/cm intestine/5 min</th>
<th>L-leucine, 20mM/cm intestine/20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Casein</td>
<td>5.3 ± .4</td>
<td>.29</td>
<td>.28</td>
<td>.29</td>
</tr>
<tr>
<td>Casein+ methionine</td>
<td>5.6 ± .6</td>
<td>.30</td>
<td>.30</td>
<td>.31</td>
</tr>
<tr>
<td>Vicia faba</td>
<td>3.8 ± .3c</td>
<td>.21</td>
<td>.20</td>
<td>.19</td>
</tr>
<tr>
<td>Vicia faba+ methionine</td>
<td>4.1 ± .5c</td>
<td>.21</td>
<td>.20</td>
<td>.19</td>
</tr>
</tbody>
</table>

^aValues listed are means for 10 rats.

^bIn vivo intestinal absorption of sugars and amino acid were carried out according to the method of Sols (1956). Five successive absorptive periods were studied in each rat.

^cDifferent (P<.01) from value for casein-fed animals.

^dLeast Significant Difference (two- and three-way ANOVA) for P<.01.
TABLE 3. *In vitro* INTESTINAL ABSORPTION OF D-GALACTOSE AND L-LEUCINE, AND OXYGEN UPTAKE BY INTESTINAL RINGS IN MALE GROWING RATS FED DIETS CONTAINING CASEIN OR RAW FIELD BEAN (*Vicia faba*) WITH OR WITHOUT ADDED METHIONINE

<table>
<thead>
<tr>
<th>Diet</th>
<th><em>In vitro</em> intestinal absorption</th>
<th>Intestinal O₂ uptake&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D-galactose (2 mM)</td>
<td>L-leucine (20 min)</td>
</tr>
<tr>
<td>Casein</td>
<td>100.0 ± 1.0</td>
<td>100.0 ± 1.0</td>
</tr>
<tr>
<td>Casein + methionine</td>
<td>105.6 ± 0.6</td>
<td>98.5 ± 0.6</td>
</tr>
<tr>
<td><em>Vicia faba</em></td>
<td>75.8 ± 5.3</td>
<td>66.5 ± 6.4</td>
</tr>
<tr>
<td><em>Vicia faba</em> + methionine</td>
<td>76.4 ± 4.9</td>
<td>71.2 ± 5.9</td>
</tr>
</tbody>
</table>

LSD<sup>e</sup> 15.6 13.9 .16

<sup>a</sup>Diets were fed *ad libitum* for 5 weeks.

<sup>b</sup>Absorption expressed as mean percentage ± SEM for 10 rats in relation to absorption of control diet (casein), which was assigned a value 100.

<sup>c</sup>Oxygen consumption ± SEM was measured by the Warburg direct method in an oxygen atmosphere at 80 oscillations/min and 3 cm amplitude in each oscillation. Incubation time, 20 minutes.

<sup>d</sup>Different (P<.01) from value for casein-fed animals.

<sup>e</sup>Least Significant Difference (two-way ANOVA) for P<.01.

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**Literature Cited**


