EVALUATION OF NUTRITIONAL STATUS OF COMMON VETCH (Vicia sativa) ON GROWING RATS

M. R. Islam1, A. M. M. Bari, M. H. Rahman and M. M. Rahman1
Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

ABSTRACT

Anti-nutritional effect of common vetch (Vicia sativa) was evaluated in 12 growing Long-Evans rats of either sex, divided into three groups (A, B & C), each consisting of 4 rats during the period from September 1999 to April 2000. Rat of group A fed with commercial poultry pellet feed (Quality Poultry Ltd. Dhaka). Group B fed feed supplemented ration and group C fed with ration contained vetch and methionine for a period of 43 days. Results showed that the body weight gain was significantly (p < 0.01) lower in rats fed with vetch supplemented (180.35 ± 11.99) g per comparison to control (220.25 ± 22.87) g and vetch + methionine supplemented (246.25 ± 25.92) g, diet. On proportionate weight gains, growth rate was significantly (p < 0.01) higher in rats fed vetch supplemented diet (167.08 ± 12.72 cm) than those of vetch + methionine supplemented (106.65 ± 4.12 cm) and control (123.53 ± 19.85) cm groups. Addition of methionine in the vetch diet resulted in significant (p < 0.01) increase in pancreatic weight on wet weight basis. The apparent digestibility of nitrogen was found to be increased in case of rats fed vetch supplemented diet than those of control and vetch + methionine supplemented diets.

Key words: Anti-nutritional effect, common vetch, growing rat

INTRODUCTION

Common vetch (Vicia sativa) is a leguminous pea-like plant, grown extensively in certain regions of the world for covering crops, hay-making and green manure. It is used as a cattle feed, but also used as a cheap substitute for lentils, as human food. Vetch bears considerable physical similarity (Tate and Eiinoke, 1992.) to those of red lentil. It is slightly larger in size and lighter in color than the latter. Vetch is very high in nitrogen. On dry weight basis (g/kg), vetch is composed of protein 38.4, fiber 84 and ash 34 (D’Helle and Devendra, 1993). On air-dry basis, mean percentage composition of vetch is nitrogen 3.56, phosphorus 0.30, potassium 1.52, calcium 1.52 and magnesium 0.30 (Klingman, 1963). The toxic isoflavon conjugate in vetch is 500-1117 mg/kg in dry matter (Lucien, 1966). Vetch contains cyanide 1.90 micrograms/g (Smith and Baharum, 1980). As a toxic principle vetch contains 1% L-beta-cyaoalanilide and 1.1% gamma-L-thiinyl derivatives (Reseler et al., 1965), which are neurotoxins. There are several reports of neurotoxicity from consumption of vetch in pigs, mule, horses, ducks, monkeys, turkeys and chicken (Tate and Eiinoke, 1992). Seeds of common vetch entered into Bangladesh food chain from South Australia officially from 1990’s as a cheap substitute for red lentils or Mosher dahl (Lens culinaris) as complementary to rice. Common vetch enjoyed popularity in a nation like ours where Dal (1) is one of the basic constituents of the meal. Rahman (1999b, 2000) reported that vetch was thinly consumed by the inmates of jail, police froces, armed forces and general public, received it as windfall because they are cheaper than the Mosher dahl. However, trouble began, when one of the Non Government Organization’s, “PROSHIKA,” began campaign against its ordinability as a food item and free access to the Bangladesh market. Although there had been no scientific evidence of toxicity in humans, however, Bangladesh Government banned import, store, sale or consumption of vetch due to PROSHIKA’s campaign from October 1999. Thus, the present investigation was undertaken to study the anti-nutritional effect in terms of vetch’s apparent digestibility and body and organ weights of the growing rats and to observe any clinical signs and symptoms or lethality, with a view to find out the toxicity of common vetch.

MATERIALS AND METHODS

This study on the effect of feeding vetch in 12 apparently healthy Long-Evans rats of either sex was carried out during the period from September 1999 to April 2000. These rats were born at the Experimental Laboratory Animals Unit of the Department of Pathology, Bangladesh Agricultural University, Mymensingh which were fed with rations fed up to 50 days of age, and then they were used in the experiment.

Copyright © 2003 Bangladesh Society for Veterinary Medicine
All rights reserved 1729-7893/01/2003

1Present address: Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh.
The initial body weights were between 100 and 110 g. These 12 rats were divided into three groups (A, B & C), each consisting of four rats. These three groups of rats were maintained in three different specially designed separate cages throughout the experimental period.

**Feed preparation**

The rats of group A (control) were supplied with commercial poultry feeds for starter and grower (Quality Feeds Ltd, Dhaka). The rats of group B (vetch supplemented) were supplied with feed supplemented with vetch powder. This feed was prepared with maize starch (26%), wheat bran (10%), glucose (15%), soybean oil (15%), powdered vetch (34.4%), lysine (0.1%), and table salt (0.5%). The rats of group C (vetch + methionine) were supplied with vetch supplemented feed as group B with adding 0.01% methionine as vetch is deficient in methionine (Georgelova, 1986). Each of the experimental rats was observed for any abnormality or lethality during the 43 days of experimental period. The body weight and feed intake were recorded and feed of all experimental rats were collected regularly.

**Killing of animals and sample collection**

After feeding for 43 days each of the 12 rats of all the three groups were killed by anesthesia with diethyl ether. An incision was made on midline from anus to throat region. The carcasses were eviscerated morphologically and weights were taken. The organs such as liver, heart, kidney, stomach, lungs, pancreas, intestines, stomach, spleen were examined grossly and collected and weighed (organ weight / 100 g total body weight). For nitrogen estimation and measurement of moisture percentage in carcasses, the carcasses were collected daily and were stored at -20°C.

**Determination of apparent digestibility of feed**

Nitrogen percentage in feeds (control, vetch supplemented and vetch + methionine supplemented) and carcasses were determined according to semi-micro Kjeldahl method (Davidson et al., 1970). Apparent digestibility was calculated on the basis of daily feed consumption, nitrogen percentage of feed and carcasses.

\[ N \text{ in per 100 g feed} = \frac{\text{Fecal N in per 100 g feed}}{N \text{ in per 100 g feed}} \times 100 \]

**Determination of absolute gut length percentage**

Absolute gut length percentage was determined on the basis of body weight change during the feeding period and length of gut. The length of gut was measured after killing and dissecting the organs with measuring scale. The body weights of rats were taken daily before feeding and during the experimental period.

\[ \text{Length of gut} \]

\[ \text{Absolute gut length percentage} = \frac{\text{Final wt (g)} - \text{Initial wt (g)}}{\text{Length of gut}} \times 100 \]

**Determination of proportional difference of organs**

The collected organs of rats of different groups were freeze-dried, then the weight of organs and carcasses were measured. Proportion of difference of organs was calculated.

\[ \text{Organ wt (g)} \times 100 \]

**Determination of moisture percentage of carcasses**

Moisture percentage of carcasses was determined from the collected carcasses of last 25 days of experimental period. Moisture was determined by oven drying the weighed samples at 70°C for 24 hours, cooled in a desiccator and weighed. The procedure was repeated until constant weight was obtained. The percent loss in weight was reported as percent moisture content.

**Statistical analysis**

The data were analysed using Student's paired 't' test with Minitab programme.

58
RESULTS AND DISCUSSION

There were no detectable clinical signs or any abnormalities recorded in any single rat in any group. The effect on body weight gain of three different groups of rats is presented in the Table 1. After feeding for 43 days, the average body weight gain in the supplemented group (183.75±11.09 g) was significantly lower (p<0.01) than those of controls (221.52±22.87 g) and vetch + melatinine supplemented groups (246.25±2.5 g). The effect on proportional difference of organs (organs/weight /100) total body weight in all groups is shown in the Table 1. Proportional wet weight of stomach in the vetch + melatinine supplemented group (0.55 ± 0.03 g) was found to be lower than those of vetch supplemented (0.72 ± 0.05 g) and control (0.73 ± 0.05 g) groups, which is statistically significant (p<0.01). On the other hand, weight of jejunum in the vetch supplemented group (3.33 ± 0.04 g) was found to be significantly higher (p<0.05) than those of vetch + melatinine supplemented (3.11 ± 0.2 g) and control groups (3.16 ± 0.17 g). Rats receiving vetch + melatinine supplemented diet (1.0 ± 0.09 g) showed a significant increase (p<0.01) in the weight of the pancreas compared with those of vetch supplemented (0.54 ± 0.06 g) and control (0.40 ± 0.21 g) groups. The wet weight of thymus was apparently increased (p<0.03) but the spleen (0.18 ± 0.05 g) was decreased significantly (p<0.05) in vetch supplemented group. Proportional wet weight of kidney in the vetch supplemented group (0.44 ± 0.04 g) was found to be slightly higher (p>0.05) than those of vetch + melatinine supplemented (0.43 ± 0.04 g) and control (0.38 ± 0.04 g) groups. Wet weight of liver and colon was found to be lower in the vetch + melatinine supplemented group than those in the control and vetch supplemented groups but in case of colon it differs significantly (p<0.05) with control. No remarkable changes were noticed in heart on weight basis.

Table 1. Effects of certain biological parameters in rats fed with vetch supplemented and unsupplemented rations

<table>
<thead>
<tr>
<th>S/N</th>
<th>Parameters</th>
<th>Unit</th>
<th>Control rats</th>
<th>Vetch supplemented</th>
<th>Vetch + melatinine supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Post-feeding body weight</td>
<td>g</td>
<td>101.25±5.02</td>
<td>107.35±0.79</td>
<td>105.00±4.08</td>
</tr>
<tr>
<td>2.</td>
<td>Body weight change</td>
<td>%</td>
<td>221.52±22.87</td>
<td>** 183.75±11.09</td>
<td>246.25±2.50</td>
</tr>
<tr>
<td>3.</td>
<td>Liver weight</td>
<td>g</td>
<td>120.03±23.45</td>
<td>** 80.02±17.17</td>
<td>141.25±4.79</td>
</tr>
<tr>
<td>4.</td>
<td>Heart weight</td>
<td>g</td>
<td>3.67±0.28</td>
<td>3.64±0.23</td>
<td>3.63±0.24</td>
</tr>
<tr>
<td>5.</td>
<td>Kidney weight</td>
<td>g</td>
<td>0.36±0.05</td>
<td>0.34±0.04</td>
<td>0.33±0.02</td>
</tr>
<tr>
<td>6.</td>
<td>Spleen weight</td>
<td>g</td>
<td>0.38±0.06</td>
<td>*0.4±0.04</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td>7.</td>
<td>Lung weight</td>
<td>g</td>
<td>0.73±0.05</td>
<td>0.72±0.05</td>
<td>*0.55±0.01</td>
</tr>
<tr>
<td>8.</td>
<td>Spleen weight</td>
<td>g</td>
<td>0.49±0.06</td>
<td>0.58±0.04</td>
<td>0.49±0.04</td>
</tr>
<tr>
<td>9.</td>
<td>Jejunum weight</td>
<td>g</td>
<td>3.16±0.17</td>
<td>*3.33±0.04</td>
<td>3.16±0.25</td>
</tr>
<tr>
<td>10.</td>
<td>Carcinoid weight</td>
<td>g</td>
<td>0.44±0.04</td>
<td>0.47±0.52</td>
<td>0.46±0.06</td>
</tr>
<tr>
<td>11.</td>
<td>Colon weight</td>
<td>g</td>
<td>0.74±0.06</td>
<td>0.63±0.02</td>
<td>*0.53±0.04</td>
</tr>
<tr>
<td>12.</td>
<td>Thymus weight</td>
<td>g</td>
<td>0.19±0.01</td>
<td>0.26±0.03</td>
<td>0.17±0.02</td>
</tr>
<tr>
<td>13.</td>
<td>Pancreas weight</td>
<td>g</td>
<td>0.50±0.05</td>
<td>0.46±0.06</td>
<td>** 0.03±0.09</td>
</tr>
<tr>
<td>14.</td>
<td>Spleen weight</td>
<td>g</td>
<td>0.27±0.04</td>
<td>*0.18±0.05</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>15.</td>
<td>Length of gut</td>
<td>cm</td>
<td>150.33±3.11</td>
<td>** 144.75±0.23</td>
<td>133.50±1.46</td>
</tr>
<tr>
<td>16.</td>
<td>Absolute gut length</td>
<td>cm</td>
<td>124.73±19.85</td>
<td>** 167.08±12.72</td>
<td>163.64±4.12</td>
</tr>
<tr>
<td>17.</td>
<td>Feed intake</td>
<td>cm</td>
<td>220±9</td>
<td>220±9</td>
<td>220±9</td>
</tr>
<tr>
<td>18.</td>
<td>Nitrogen / 100 g feed</td>
<td>%</td>
<td>6.8</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>19.</td>
<td>Nitrogen / 100 g feed</td>
<td>%</td>
<td>2.34</td>
<td>2.15</td>
<td>3.69</td>
</tr>
<tr>
<td>20.</td>
<td>Fecal nitrogen/100 g feed</td>
<td>%</td>
<td>0.47</td>
<td>3.15</td>
<td>0.33</td>
</tr>
<tr>
<td>21.</td>
<td>Apparent digestibility</td>
<td>%</td>
<td>82.46</td>
<td>91</td>
<td>88.5</td>
</tr>
<tr>
<td>22.</td>
<td>Fecal weight (dry)</td>
<td>g</td>
<td>801.41*</td>
<td>244.4*</td>
<td>387.29*</td>
</tr>
<tr>
<td>23.</td>
<td>Fecal weight (dry)</td>
<td>g</td>
<td>482.49</td>
<td>144.76*</td>
<td>205.19*</td>
</tr>
<tr>
<td>24.</td>
<td>Moisture</td>
<td>%</td>
<td>39.71</td>
<td>40.76</td>
<td>47.53</td>
</tr>
</tbody>
</table>

*Indicates significant (p<0.05); **Indicates significant (p<0.01). *Data approved for last 25 days of period of experiment.

Absolute gut length per 100 g live body weight is also shown in the Table 1. Absolute gut length percentage significantly (p<0.01) increased in vetch supplemented (melatinine unsupplemented) group (140.08 ± 12.72 g) in

59
comparison with those in vert - methionine supplemented ( 106.6 ± 4.12 g ) and control ( 123.5 ± 19.3 g ) groups. Gut length per 100 g live body weight of vert - supplemented ( methionine unsupplemented ) group appeared to be 1.5 times higher than that of the vert- methionine supplemented group. Result on apparent digestibility is also presented in the Table 1. Nitrogen intake by rats was much higher in control group compared to vert + methionine supplemented and vert supplemented groups. Fecal nitrogen was higher in case of vert + methionine supplemented ( 3.69 g ) group compared to unsupplemented ( 2.35 g ) and controls ( 2.54 g ). However, apparent digestibility was increased in rats of vert supplemented ( methionine unsupplemented ) group ( 91% ) than those of control ( 82.46% ) and vert + methionine supplemented ( 88.5% ) groups Moisture content of faeces is also shown in the Table 1. The moisture content of faeces was apparently higher in rats of methionine supplemented group ( 47.53% ) compared to control ( 39.79% ) and methionine unsupplemented ( 40.76% ) groups. The present report describes some preliminary anti-nutritional and clinico-pathological effects of Vicia sativa feeding in rats. The aim of the work was to resolve the national issue over the use of vert as an exclusive source of protein for animals. After their brief news flash, in Non Government Organization ( NGO ) persuaded the Government to ban its sale or consumption in this country. The eventual public ban was put into effect from October 1999. However, there had been no report of overt toxicity from vert in the history like lablabus from Bangladesh or elsewhere and there were no clinical signs of neoplastic disorders as reported in pigs by Tate ( 1997 ). The most frequently used method of expressing nutritional status in the present experiment was to see the change of weight with an increase in age. Since both vert supplemented and vert + methionine supplemented groups achieved weight gains, it may be concluded that the nutritional status of either group did not differ in the results of the two test diets for 3 days had no adverse effect on the capacity of the animal to gain weight. However, compared to those of vert supplemented ( methionine unsupplemented ) group, rats' Bsd on supplemented with methionine gained almost double live weight in the above time. The low level of sulfur containing amino acids and tryptophan found in legumes are well - established fact ( Coussens et al., 1969 ) for rats. Thus amino acid deficiencies could have, as previously shown ( Gumbman and Friedman, 1987 ) , greatly limited the extent to which rats could utilize vert protein. Protein Efficiency Ratio ( PER ) values obtained with diets containing raw vert were however, much poorer than expected from the feeding data. Also, supplementation of the diet with methionine had indeed no effect on the feed consumption. However, there was a substantial increase in the body weight gain. Thus, although supplementation with methionine could not increase the feed intake, supplementation appears to be the main factor increasing the efficiency of vert protein utilization by rats. The faecal nitrogen output of vert-fed rats was considerably higher than the corresponding controls. Faecal nitrogen could be derived from a number of diverse sources such as undigested dietary protein, digestive enzymes, mucin, shedding of gut cells, bacteria and leakage of serum proteins in the gut lumen. The presence of inhibitor substances and partial resistance of vert proteins to proteolytic degradation are likely to lead to increase amounts of dietary protein surviving passage and to an increased secretion of proteolytic enzymes ( Glenn and Leyman, 1973 ) . In addition, faecal nitrogen has been reported to interfere with normal gut flora in some species ( Chapman, 1986 ) and also cause hyperammonia type reactions in the gut ( Miller et al., 1984 ) that would lead to increase mucosa production, cell turnover and leakage of serum proteins. Therefore, the high nitrogen output found could have been due to increases in some or all of the potential sources of faecal nitrogen. However, despite these changes, nearly, the vert-fed rats apparently absorbed 90% of the dietary nitrogen. There was a significant increase in both gut weight and length of the small intestine of rats fed on raw vert diets. A similar effect was previously observed in calves when fed raw soybean diet ( Roy et al., 1977 ) . Enhancement of the intestinal growth and weight has been linked to stimulation of protein or other growth factors ( Klein and McKenzie, 1983 ) . The total food intake of rats fed vert raw was not found to be reduced than the vert + methionine supplemented group, however the small intestine was enlarged compared with that of the rats on vert + methionine supplemented diet. It is therefore, unlikely that enlargement was to any significant extent links to changes in intestinal nutrition or dietary mass. The schematic weight increased considerably in rats fed vert + methionine supplemented diet. This enlargement has been generally attributed to the effects of trypsin inhibitors ( Ory, 1981 ) . However, a few workers ( Naim et al. 1982 ) have also shown that soybean fractions of low tryptic inhibitory activity have also been shown to cause significant enlargement of the pancreas. Therefore, other dietary components may also contribute to the overall increase in pancreas size. Pancreatic enzymes such as trypsin and chymotrypsin contain a greater than usual proportion of sulfur containing amino acids, approximately 6.7% ( Bernhard, 1968 ) . Therefore, any increase in enzyme production would increase the demand for sulfur containing amino acids. This may also have adverse effects on the overall utilization of absorbed amino acids and lead to an increase in urea excretion.
The overall growth achieved per gram of nitrogen retained by rats was considerably lower for those fed diets containing raw vetch than those of supplemented feed. This difference would have occurred if the rats of lipid, glycogen, and/or water accumulation were lower than those in control rats. Since no malabsorption of these components was apparent, this suggests that there was increased catabolism of glycogen and/or lipid. If there was increased utilization of these components as a result of raw vetch feeding, this may have also resulted in a higher rate of catabolism of amino acids and thus a higher urinary area output. Diarrhea did not occur in the rats on the test diets since the moisture content of the facers of the rats on either diet was nearly same.

Demonstration of comparatively higher proportional weights of the kidneys in rats fed on vetch diets than the controls indicates that Vicia sativa might have some unknown anti-nutritional factors that involve the kidneys. Although, the urinary output data on probable nephrotoxic agents could not have been determined from the vetch.

REFERENCES
18. Reschi C, Nisan N, Guen YA and Rotv J (1983). Isolation and identification from common vetch of 4-p-(chlorophenyl)-5-uroso-