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EVALUATION OF NUTRITIONAL STATUS OF COMMON VETCH (*VICIA SATIVA*) ON GROWING RATS

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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. Rats of group A fed with commercial poultry pellet feed (Quality Feeds Ltd., Dhaka), group B fed vetch 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 (183.75 ± 11.09 g) in comparison to control (221.25 ± 22.87 g) and vetch + methionine supplemented (246.25 ± 2.5 g) diets. On proportional weight basis, gut length was significantly ($p < 0.01$) higher in rats fed vetch supplemented diet (167.08 ± 12.72 cm) than those of vetch + methionine supplemented (106.63 ± 4.12 cm) and control (123.53 ± 19.85 cm) groups. Addition of methionine in the vetch diet resulted in a significant ($p < 0.01$) increases 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 diet.

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 cattle feed, but also used as a cheap substitute for lentils, as human food. Vetch bears considerable physical similarity (Tate and Enneking, 1992) to those of red lentil. It is slightly larger in size and lighter in color than the later. Vetch is very high in nitrogen. On dry weight basis (g / kg) vetch is composed of protein 304, fiber 84 and ash 34 (D'Mello and Devendra, 1995). On air-dry basis, mean percentage composition of vetch is nitrogen 3.58, phosphorus 0.30, potassium 1.52, calcium 1.52 and magnesium 0.30 (Klingman, 1961). The toxic manganese concentration in vetch is 500-1117 mg / kg in dry matter (Luxmoore, 1991). Vetch contains cyanide 1.90 micromoles / g (Smith and Bababunms, 1980). As a toxic principle vetch contains 0.1% L-beta-cyanoalanine and 1.1% gamma L-glutamyl derivatives (Ressler *et al.*, 1963), which are neurotoxins. There are several reports of neurotoxicity from consumption of vetch in pigs, mules, horses, ducks, monkeys, turkeys and chickens (Tate and Enneking, 1992). Seeds of common vetch entered into Bangladeshi food chain from South Australia officially from 1990's as a cheap substitute of red lentil or Moshur dahl (*Lens culinaris*) as complementary to rice. Common vetch enjoyed popularity in a nation like ours where Dahl is one of the basic constituents of the manue. Rahman (1999ab, 2000) reported that vetch was mainly consumed by the inmates of jail, police forces, armed forces and general public, received it as windfall because they are cheaper than the Moshur dahl. However, trouble begun, when one of the Non Government Organization's, "PROSHIKA" begun campaign against its credibility as a food item and free access to the Bangladeshi 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 rational feed up to 50 days of age, and then they were used in the experiment.

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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 (33.4%), 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 of methionine (Gergelyiova, 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 faeces 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 examined morphologically and weights were taken. The organs such as liver, heart, kidney, stomach, lungs, jejunum, cecum, colon, thymus, pancreas and spleen were examined grossly and collected and weighed (organ weight / 100 g total body weight). For nitrogen estimation and measurement of moisture percentage in faeces, the faeces were collected daily and were stored at -20^0C.

Determination of apparent digestibility of feed

Nitrogen percentage in feeds (control, vetch supplemented and vetch + methionine supplemented) and faeces 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 faeces.

$$\text{Apparent digestibility} = \frac{\text{N in per 100 g feed} - \text{Faecal N in per 100 g feed}}{\text{N in per 100 g feed}} \times 100$$

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.

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

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. Proportional difference of organs was calculated.

$$\text{Proportional difference} = \frac{\text{Organ wt (g)}}{\text{Total body wt (g)}} \times 100$$

Determination of moisture percentage of faeces

Moisture percentage of faeces was determined from the collected faeces of last 25 days of experimental period. Moisture was determined by oven drying the weighed samples at 70^0C 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 analyzed using Student's paired 't' test with Mstat-C programme.

RESULTS AND DISCUSSION

There were no detectable clinical signs or any abnormality 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 vetch supplemented group (183.75±11.09 g) was significantly lower ($p < 0.01$) than those of control (221.25±22.87 g) and vetch + methionine supplemented groups (246.25 ± 2.5 g). The effect on proportional difference of organs (organs weight / 100 g total body weight) in all groups is shown in the Table 1. Proportional wet weight of stomach in the vetch + methionine supplemented group (0.55 ± 0.03 g) was found to 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, wet 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 + methionine supplemented (3.11 ± 0.25 g) and control groups (3.16 ± 0.17 g). Rats receiving vetch + methionine supplemented diet (1.03 ± 0.09 g) showed a significant increase ($p < 0.01$) in the wet weight of the pancreas compared with those of vetch supplemented (0.54 ± 0.06 g) and control (0.60 ± 0.21 g) groups. The wet weight of thymus was apparently increased (0.23 ± 0.03 g) but the spleen (0.18 ± 0.005 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 + methionine supplemented (0.43 ± 0.04 g) and control (0.38 ± 0.06 g) groups. Wet weight of liver and colon was found to be lower in the vetch + methionine 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 wet weight basis.

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

S/N	Parameters	Unit	Control rats	Vetch supplemented	Vetch + methionine supplemented
1.	Pre-feeding body weight	g	101.25±02.50	103.75±04.79	105.00±4.08
2.	Post-feeding body weight	g	221.25±22.87	** 183.75±11.09	246.25±2.50
3.	Body weight change	g	120.00±23.45	** 80.00±8.17	141.25±4.79
4.	Liver weight	g	3.67±0.28	3.64±0.25	3.46±0.34
5.	Heart weight	g	0.36±0.05	0.34±0.04	0.33±0.02
6.	Kidney weight	g	0.38±0.06	*0.44±0.04	0.43±0.04
7.	Stomach weight	g	0.73±0.05	0.72±0.05	**0.55±0.03
8.	Lung weight	g	0.49±0.06	0.58±0.04	0.49±0.06
9.	Jejunum weight	g	3.16±0.17	*3.33±0.04	3.11±0.25
10.	Caecum weight	g	0.44±0.04	0.47±0.02	0.41±0.06
11.	Colon weight	g	0.74±0.09	0.63±0.02	*0.53±0.04
12.	Thymus weight	g	0.19±0.02	0.23±0.03	0.17±0.02
13.	Pancreas weight	g	0.60±0.21	0.54±0.06	** 1.03±0.09
14.	Spleen weight	g	0.27±0.04	*0.18±0.005	0.21±0.02
15.	Length of gut	cm	144.75±05.23	133.50±14.66	150.50±3.11
16.	Absolute gut length/ 100g live body weight	cm	123.53±19.85	** 167.08±12.72	106.63±4.12
17.	Feed intake	g	2405#	2215#	2220#
18.	Nitrogen / 100 g feed	%	2.68	1.68	2
19.	Nitrogen / 100 g faeces	%	2.34	2.35	3.69
20.	Fecal nitrogen/100g feed	%	0.47	0.15	0.33
21.	Apparent digestibility	%	82.46	91	88.5
22.	Faecal weight (wet)	g	801.61#	244.4#	387.19#
23.	Faecal weight (dry)	g	482.9#	144.76#	203.15#
24.	Moisture	g	318.75	99.64	184.04
25.	Moisture	%	39.75	40.76	47.53

*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 (methionine unsupplemented) group (167.08 ± 12.72 g) in comparison with those in vetch + methionine supplemented (106.63 ± 4.12 g) and control (123.53 ± 19.85 g) groups. Gut length per 100 g live body weight of vetch supplemented (methionine unsupplemented) group appeared to be 1.5 times higher than that of the vetch + methinine 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 vetch + methionine supplemented and vetch supplemented groups. Fecal nitrogen was higher in case of vetch + methionine supplemented (3.69 g) group compared to unsupplemented (2.35 g) and controls (2.34 g). However, apparent digestibility was increased in rats of vetch supplemented (methionine unsupplemented) group (91%) than those of control (82.46%) and vetch + 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 (39.75%) 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 vetch as an exclusive source of protein for mammals. After their brief news flash, a 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 vetch in the history like lathyrus from Bangladesh or elsewhere and there were no clinical signs of neuropathological 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 vetch supplemented and vetch + methionine supplemented groups achieved weight gains, it may be concluded that the nutritional status of either group or feeding of the two test diets for 43 days had no adverse effect on the capacity of the animal to gain weight. However, compared to those of vetch supplemented (methionine unsupplemented) group, rats' fed on supplemented with methionine gained almost double live weight in the above time. The low level of sulfur containing amino acids and tryptophans found in raw legumes are well-established fact (Coates *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 vetch protein.

Protein Efficiency Ratio (PER) values obtained with diets containing raw vetch 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 vetch protein utilization by rats.

The faecal nitrogen output of vetch-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, mucus, shedding of gut cells, bacteria and leakage of serum proteins in the gut lumen. The presence of inhibitor substances and partial resistance of vetch proteins to proteolytic degradation are likely to lead to increase amounts of dietary protein surviving gut passage and to an increased secretion of proteolytic enzymes (Green and Lyeman, 1972). In addition, legumes have been reported to interfere with normal gut flora in some species (Hampson, 1986) and also cause hypersensitivity-type reactions in the gut (Miller *et al.*, 1984) that would lead to increase mucus 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 vetch-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 vetch diets. A similar effect was previously observed in calves when fed raw soybean diet (Roy *et al.*, 1977). Enlargement of the intestine would occur in response to increase luminal nutrition of dietary mass or to stimulation by hormone or growth factors (Klein and McKenzie, 1983). The food intake of rats fed raw vetch was not found to be reduced than the vetch + methionine supplemented group, however the small intestine was enlarged compared with that of the rats on vetch + methionine supplemented diet. It is therefore, unlikely that enlargement was to any significant extent links to changes in luminal nutrition or dietary mass.

The pancreatic weight increased considerably in rats fed vetch + 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 trypsin 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 rates 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 urea output. Diarrhea did not occur in the rats on the test diets since the moisture content of the faeces 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 or probable nephrotoxic agents could not have been determined from the vetch.

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