HAEMATO - BIOCHEMICAL AND HISTOLOGICAL CHANGES UPON SUPPLEMENTED EXTRUDED FABA BEAN *(VICIA FABA L.)* FEEDING IN RATS

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ABSTRACT

Supplementation of extruded faba bean (EFB) in cereals diet as a protein source improved the content of RBC, PCV and haemoglobin in weaning albino rats and decreased cholesterol level No severe deleterious effect on the structural components of parenchymatous tissues was observed; architectural pattern was well maintained. Higher supplementation of EFB at 40% in diet did not prove beneficial and effective as compared to its 20% supplementation and therefore, not recommended.

KEY WORDS: Extruded faba bean, Supplementation, Haemato -biochemical, Histology, Rats

INTRODUCTION

Faba bean *(Vicia faba L.)* is a newly introduced crop in Indian agriculture and is being advocated as a substitute after soybean for its high protein content by nutrition and health experts. Extrusion cooking, a modern high temperature short time technology, increased its protein content. (Rajawat et al., 2000). It also proved to be useful in eliminating some toxic anti-metabolites (Rajawat et al., 1999). Extruded faba been (EFB) protein was utilized in a better way by rats when EFB was supplemented at 20 % level in a cereal based diet (Kushwah et al. 2002). Observations on certain hepatic clinical enzymes, mainly those associated with protein metabolism, also indicated efficient amino acids availability of dietary protein at low level for proper utilization (Rajawat et al., 2002). In addition, it also indicated hepato-toxic effect of EFB when supplemented at 40% level that put the animals under stress owing to low utilization of dietary protein affecting nitrogen metabolism (Rajawat et al., 2002). The present investigation is an extension to the above referred work. It was deemed worthwhile to assess the haemato-biochemical as well as histological changes in some organs of rats for establishing the usefulness of EFB as a protein supplement in cereal based diet.

MATERIALS AND METHODS

Extrusion conditions : Pure seeds of three varieties of faba bean *Vicia faba* L. namely, JV-1, JV-2 and JV-3 were procured from the Department of Plant Breeding and Genetics, Jawaharlal Nehru Krishi Vishwa Vidayalaya, Jabalpur, M.P., India. These were dehulled separately, ground in a Mini Dal Mill and sieved to obtain uniform particle size. The flour of all the three varieties was analyzed for proximate composition and anti-nutritional components and mixed in equal ratio to over-rule inter-varietal differences (Rajawat et al., 1999 and 2002). The mixed flour was then extruded on WENGER X-5 model at extrusion temperature of 75°C and feed moisture of 20% keeping screw speed of 400 rpm and a residence time of 5 to 10 sec constant. At this combination, faba bean flour exhibited minimal anti-nutritive effect with satisfactory nutritional quality (Rajawat et al., 1999 and 2000). For formulation of biological diets, the extrudate was air-dried, ground and mixed with cereals. Cereals, namely maize (Zea mays), kodo (Pasplum scrobiculatum) and kutki (Panicum miliare), being the staple diet were procured from the local market and analyzed for their proximate composition (Kushwah et al. 2002). These were mixed in equal ratio (1:1:1) before utilizing for preparation of different diets. The ingredient composition of various experimental diets is shown in Table

Animals: Twenty four colony bred weaning albino rats, 21-day old, weighing 25 to 35 g of either sex, were procured from the animal colony of College of Veterinary Science and Animal Husbandry, Mhow, M.P. India and were divided into four groups of six each and housed in individual cages. These were allowed to feed on variable experimental diets as detailed in Table 1 and were given water *ad libitum.* Casein, a biologically active protein source formed the standard diet. The other diets constituted cereals alone and cereals supplemented with either 20% or 40% extruded faba bean (EFB). The level of blending of EFB was established on basis of organoleptic testing of EFB blended cereal recipes (Rajawat et al. 2002). Before feeding to animals, all the diets were steamed for 15 min. All diets were nutritionally complete at 10% protein level (AOAC, 1980)

containing fat (refined groundnut oil) vitamin-mineral mixture, crude fiber (non-nutritive cellulose) and starch (Kushwah et al., 2002)

: 1 – Composition of experimental diets (Isoproteinic at 10%)

nstituents (g/100g)	Gr-I Casein	Gr.II Cereals ** based diet	Gr.III Cereals +20% EFB *	Gr.IV Cereals +40% EFB *
ein source	10.31	100	69.56	53.33
ml/100g of diet)	6.59	-	6.33	6.98
nin+ Mineral Mixture	0.60	-	0.60	0.60
le fibre	1.00	-	1.00	1.00
ch	81.50	-	22.15	38.09
l	**100.00**	**100.00**	**100.00**	**100.00**

uded Faba Bean ** Kodo+Kutki+ Maize

Haemato-biochemical study: The feeding trial lasted for a period of 28 days. On the next day of the feeding trial, animals were sacrificed upon decapitation. The blood of each animal was collected separately in vials containing EDTA and tested for haemoglobin, total erythrocyte count (TEC), total leucocyte count (TLC), packed cell volume, erythrocytic indices namely mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) by standard methods (Sachlm et al., 1975). Samples were also tested for total cholesterol and total proteins by using commercial diagnostic kits (Span Diagnostic Ltd., Surat, India.)

Histological study: Upon sacrifice, organs viz, liver, kidney, spleen and intestine were quickly excised and fixed in 10% neutral formalin. After fixation for 3-4 days, the tissues were cleaned and 2 mm thick slices from the organs were processed as per standard method and slides prepared (Luna, 1968). The prepared sections were examined for histo-pathological changes at X80, X100 or X 200 magnifications.

Statistical analysis: The data were subjected to analysis of variance after calculating mean and standard error using conventional formulae (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION:

Haemato-biochemical study:

Cereals fed rats depicted a marked reduction in the number of RBC, affecting thereby PCV and haemoglobin content as compared to casein dietary group. Total protein was also reduced whilst cholesterol content increased (Table2). Addition of 20% extruded faba bean in cereals diet, as a protein supplement, improved these values considerably and approached near to those of casein dietary group. However, its 40% supplementation in diet could not improve these values proportionately. This may be ascribed to the presence of thermostable aglycons, vicine and convicine that might have not been destroyed under specified extrusion conditions. Incomplete destruction of anti-metabolites may also add to the situation (Rajawat et al. 1999). Similar effect was also indicated in animals maintained on kodo, kutki and maize diet alone implying thereby, capability of millet protein to induce deleterious effect on normal erythropoiesis of rats as was reported earlier (Verma et al., 2001). More over, the values of erythrocytic indices remained unchanged in response to varying dietary treatments (Table -3). No plausible explanation could be made on such behavior in rats.

Compared to casein fed animals, a significant hypocholesterolemic effect was recorded in EFB blended dietary group whilst unsupplemented cereals group indicated hypercholesterolemic effect (Table 1). Faba bean protein concentrates are well known to reduce serum cholesterol level in rats (Adsule and Akpapunam 1996) and in humans too. (Fruhbeck et al., 1997).

Histological study:

Casein fed animals maintained architectural pattern of liver without degenerative or necrotic changes indicated its normal metabolic activity that was further substantiated by virtually normal haemato-biochemical profile (Table-2).

2 . Changes in haemato-biochemical values in weaning rats maintained variable dietary regimens.

	Dietary regimens			
ameters	Casein	Cereals	Cereals+ 20% EFB	Cereals+ 40% EFB
C. ($X10^6/\mu l$	$8.01^{a} \pm 0.20$	$5.83^{b} \pm 0.30$	$7.01^{a} \pm 0.11$	$7.21^{c} \pm 0.03$
C ($X10^3/\mu l$	$6.04^{ab} \pm 0.20$	$5.14^{b} \pm 0.03$	$5.83^{ac} \pm 0.18$	$5.57^{c} \pm 0.15$
noglobin (gm/dl)	$15.26^{a} \pm 0.21$	$11.80^{b} \pm 0.35$	$14.95^{ac} \pm 0.06$	$14.43^{c} \pm 0.24$
/. (%)	$48.50^{a} \pm 0.50$	$34.00^{b} \pm 0.70$	$45.00^{c} \pm 0.09$	$44.00^{c} \pm 0.40$
protein (gm/dl)	$7.61^{a} \pm 0.15$	$6.411^{b} \pm 0.30$	$7.03^{ac} \pm 0.20$	$6.86b^{c} \pm 0.25$
nin (gm/dl)	$5.85^{a} \pm 0.20$	$4.25^{b} \pm 0.30$	$5.13^{c} \pm 0.25$	$4.75^{bc} \pm 0.03$
ulin (gm/dl)	$1.76^{a} \pm 0.12$	$2.16^{a} \pm 0.20$	$1.90^{a} \pm 0.30$	$2.11^{a} \pm 0.03$
esterol (mg/dl)	$38.83^{a} \pm 0.94$	$43.33^{b} \pm 0.95$	$31.16^{c} \pm 1.50$	$33.83^{c} \pm 2.03$

s are mean ± SE of six observations;)

s with different superscripts in a row are significantly different (P<0.05)

3 . Changes in erythrocytic indices in blood of weaning rats maintained variable dietary regimens.

	Dietary regimens			
rameters	Casein	Cereals	Cereals+ 20% EFB	Cereals+ 40% EFB
V (cu.u)	$60.6^{a} \pm 1.30$	$58.90^{a} \pm 3.03$	$56.88^{a} \pm 1.20$	$61.00^{a} \pm 3.70$
H (pg)	$19.07^{a} \pm 0.44$	$20.55^{a} \pm 3.43$	$18.91^{a} \pm 0.33$	$20.01^{a} \pm 0.45$
HC (%)	$31.48^{a} \pm 0.30$	$34.76^{b} \pm 1.20$	$33.28^{ab} \pm 0.06$	$31.80^{ab} \pm 0.60$

ues are mean ± SE of six observations;)

ues with different superscripts in a row are significantly different (P<0.05)

In cereals fed group, liver showed mild fatty changes. Cereals alone could produce a deficiency of essential ingredients and thus such animals would be more prone to liver damage and its consequences. This deficiency could not be suppressed even by adding legume proteins derived from raw or germinated soybean (Gupta 1990) or horse gram (Diwakar 1994). A tendency towards degenerative changes also indicated that some toxic metabolites may be transported from the intestine to liver resulting in these changes. The presence of definite necrosis indicated toxic metabolites capable of producing death of liver cell However, the presence of some regenerative hyperplastic cells reflected biological defense mechanism that may tend to repair the damage.

Not much histological changes were observed in cellular structure of the kidneys of rats fed on variable dietary regimens except in those fed on 40% EFB blended diet. In this group, the glomeruli were predominantly observed to be with bowman's space indicating increased glomerular activity. The presence of dilatation in the renal tubules indicated some back pressure. The colloid like material producing a pseudothyroid appearance was indicative of leakage of protein like substances from the glomeruli into tubules and finally in urine.

In all sections of spleen and intestine, no marked changes were observed. Spleen was normal looking having intact capsule with well demarcated cortex, malphigian corpuscles and normal blood except in the rats raised on cereals diet consisting of maize and minor millets, kodo and kutki, where the pulp stood prominent and showed presence of haemosidrin pigment suggestive of increased haemolysis. The normal structure of intestine constituting normal muscle layer, mucous membrane and secretary protein of mucosa indicated that the diets given contained no factors that could interfere with the structure and function of intestine. Though prominent villi were observed in both EFB blended groups yet protein from extruded EFB seemed to have no severe deleterious effect on its structural components.

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