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Biological evaluation of protein quality of wheat as affected by insect infestation

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Protein quality of wheat grains having 25%, 50% and 75% infestation caused by mixed populations of *Trogoderma granarium* Everts and *Rhizopertha dominica* Fabricius was biologically evaluated by rat growth and nitrogen balance studies. Feeding of a diet containing insect-infested wheat grain (50% and 75%) resulted in marked decreases of food intake, body weight gain, food efficiency ratio, protein efficiency ratio, nitrogen absorption, biological value, net protein utilization and dry matter digestibility. These parameters showed negative association with infestation levels. However, a 25% level of grain infestation did not affect the parameters significantly.

INTRODUCTION

Wheat is an important and cheap source of protein, carbohydrates, B-vitamins and some minerals. During varied storage conditions in rural areas, wheat grains are attacked by a large number of insect pests (Atwal, 1976; Dass, 1977). Among these, *Trogoderma granarium* Everts and *Rhizopertha dominica* Fabricius are the most serious and of wider occurrence in stored grains in tropical and subtropical regions of Asia and Africa (Girish *et al.*, 1975; Atwal, 1976; Viljoen, 1990).

Chemical analysis of insect-infested cereal grains has revealed substantial losses of nutrients like carbohydrates, vitamins and minerals (Daniel *et al.*, 1977; Sharma *et al*, 1979; Jood, 1990). Insect infestation was reported to increase crude protein, non-protein nitrogen and uric acid in infested grains (Swaminathan, 1977; Hira *et al.*, 1988) but actually the true protein content of such grains has been reported to decrease (Girish *et al.*, 1975; Pushpamma & Reddy, 1979; Jood, 1990). Hence the real nutritional value of protein is not reflected in chemical analysis because of a number of factors such as presence of toxic substances, insect excreta and body fragments, proteinase inhibitors, etc., which are not accountable in chemical analysis. In such a situation, biological evaluation of protein quality is most desirable. This paper reports the effects of different levels of grain infestation (25%, 50% and 75%) caused by a mixed population of *T. granarium* and *R. dominica* on protein quality through rat growth and nitrogen balance studies.

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MATERIALS AND METHODS

Preparation of grain samples

Mass culture of two insect species (*T. granarium* and *R. dominica*) was maintained in the ambient laboratory temperature (28–39°C) and relative humidity (60–90%) conditions. The grains of a commonly consumed variety (WH-147) of wheat, apparently free from insect infestations, were procured and further subjected to aluminium phosphide fumigation to eliminate any untraced insect population. After fumigation, the grains were put in 36 glass jars (20 cm × 15 cm), each containing 1·5 kg grains. The jars were covered with muslin cloth with the help of elastic bands and placed in the laboratory for 10 days for conditioning of the grains. On the tenth day, the moisture level of the grains ranged from 10% to 11% which is congenial for multiplication of both insect species (Pingale & Girish, 1967). The jars containing grains were grouped into three sets.

In the first set of grains (nine jars), 60 larvae of *T. granarium* per jar were released to obtain three levels of infestation (25%, 50% and 75% in three replicates). In a second set 60 adults of *R. dominica* were released, while in a third set of mixed population of both species

(30 larvae of *T. granarium* + 30 adults of *R. dominica*) were released to achieve three infestation levels in three replications. In addition to this, in each set, controls (jars without insects) were kept simultaneously to compare the results with uninfested grains. To achieve the desired infestation levels, grain samples (500 grains per jar) were inspected twice a week after the release of insects; the grains which showed signs of insect damage were considered as infested and these grains were used to calculate the infestation percentage. The observation frequency was increased at later stages to ensure 25%, 50% and 75% grain infestations. It took 1, 2 and 4 months to obtain 25%, 50% and 75% levels of infestation, respectively, at ambient laboratory conditions. On the day that desired levels of infestation were actually achieved, grains were immediately disinfested with aluminium phosphide fumigation to prevent further damage and also to kill the insect population. At the end of each experimental period, control grains were not subjected to fumigation as they were free from insect infestations due to initial exposure to aluminium phosphide. After fumigation at each infestation level, grains were cleaned by passing through 4 mesh sieve to separate insects and frass (dust) from grains (Sharma *et al.*, 1979). This process ensured the removal of all frass and insect remains from infested grains. Control grains were found free from frass and insect fragments during sieving. Grains after cleaning were powdered in a Cyclotec (Tecator, Sweden) mill to pass through a 60 mesh sieve and then stored in air-tight polyethylene bottles for further chemical analysis.

Test animals

Young white male albino Wistar rats weighing 25 ± 5·0 g were obtained from the Disease and Germ Free Small Animal House, Haryana Agricultural University, Hisar, India. The rats were randomly divided into five groups, each consisting of eight rats. The rats were housed individually in polypropylene cages kept in an air-conditioned room maintained at 22 ± 1°C with a 12 h light and dark cycle. Food and water were given *ad libitum*.

Composition of diets

Composition of the diets which contained uninfested and infested wheat flour, so as to provide protein at 8% level, is given in Table 1. One group of the rats was fed on a synthetic diet containing casein as the source of protein at the 8% level. This group served as a standard check. For the preparation of the diets, ingredients were homogenized and passed through a 60 mesh sieve to ensure uniform distribution of vitamins and minerals.

The diets were prepared for one week at a time and kept in the refrigerator. All the diets were analyzed for their moisture and protein content (AOAC, 1980).

Growth experiment

Protein efficiency ratio (PER) was determined by the method of Chapman *et al.* (1959). The rats were initially weighed and then weighed on every alternate day. The weighed diet was given daily and the unconsumed diet was collected, dried and weighed. Food and water were given *ad libitum*. The rats fed on different experimental and control diets were finally weighed after four weeks and the gain in weight of rats during this period was recorded. The amounts of food and protein intake during this period were calculated on a dry matter basis. Food efficiency ratio (FER) and PER were calculated by the following formulae:

$$\text{PER} = \frac{\text{gain in body weight (g)}}{\text{protein consumed (g)}}$$

$$\text{FER} = \frac{\text{gain in body weight (g)}}{\text{food consumed (g)}}$$

Nitrogen balance studies

For nitrogen balance studies, adult male Wistar rats

Table 1. Composition of the experimental diets (g/kg diet)

Ingredients	Casein	Wheat			
		Uninfested	Infested		
			25%	50%	75%
Casein	100	—	—	—	—
Flour	—	678·0	701·8	727·9	785·6
Sucrose	50	50	50	50	50
Groundnut oil	100	85·8	85·8	85·7	86·5
Mineral mixture	40	40	40	40	40
Vitamin mixture	10	10	10	10	10
Cellulose	50	—	—	—	—
DL-Methionine	3·0	—	—	—	—
Choline bitartrate	2·00	2·00	2·00	2·00	2·00
Corn starch	645·0	134·2	110·7	84·4	45·9

were housed individually in polypropylene metabolic cages. One group of rats was fed on a protein-free diet and another on a casein diet. Four groups of rats were fed on test diets (containing flours of grains showing 0%, 25%, 50% and 75% insect infestations). The experiment was conducted for nine days, which included an initial conditioning period of four days. During the last five days, urine and faeces from each rat were collected separately. Food and water were given *ad libitum* and the change in body weight was recorded. The concentration of nitrogen in urine and faeces was estimated by the microKjeldahl method (AOAC, 1980). The data obtained from this experiment were used to calculate apparent digestibility (AD), true digestibility (TD), biological value (BV) (Chick *et al.*, 1935), net protein utilization (NPU) (Platt *et al.*, 1961), net protein retention (NPR) and protein retention efficiency (PRE) (Bender & Doell, 1957), dry matter digestibility (DMD) and utilizable proteins (UP) (Gupta *et al.*, 1979) by employing the following formulae:

$$AD = \frac{Ni - NF_1 \times 100}{Ni}$$

$$TD = \frac{Ni - (NF_1 - NF_2) \times 100}{Ni}$$

$$BV = \frac{Ni - (NF_1 - NF_2) - (NU_1 - NU_2) \times 100}{Ni - (NF_1 - NF_2)}$$

Ni = nitrogen intake of animals fed test diet

NF_1 = nitrogen excreted in faeces of animals fed test diet

NF_2 = nitrogen excreted in faeces of animals fed protein-free diet

NU_1 = nitrogen excreted in urine of animals fed test diet

NU_2 = nitrogen excreted in urine of animals fed protein-free diet

$$NPU = \frac{BV \times TD}{100}$$

$$NPR = \frac{\text{weight gain of test group + weight loss of protein-free group}}{\text{weight of test protein consumed}}$$

$$PRE = NPR \times 16$$

$$UP = \frac{NPU \times N\ (\%\ \text{of dry matter}) \times 6{\cdot}25}{100}$$

$$DMD = \frac{\text{food consumed} - \text{faecal weight}}{\text{food consumed}} \times 100$$

Statistical analysis

The data were subjected to analysis of variance (ANOVA) in a completely randomized design to determine the significant differences among various treatments (Snedecor & Cochran, 1968).

RESULTS AND DISCUSSION

Food efficiency ratio and protein efficiency ratio

Food intake on the casein diet (control) was the highest (228·45 g per rat) and it differed significantly ($p \leq 0{\cdot}05$) from that of other diets (Table 2). Consumption of the diet containing flour of 75% infested grains was significantly lower than that of uninfested grains. There was a progressive decrease in food intake with the increase of infestation levels. Diets containing infested grains may not be palatable to rats due to the presence of uric acid, antinutrients and rancidity (Jood, 1990), thereby leading to poor food intake. Pant and Susheela (1977) also recorded poor food intake of insect-infested sorghum grains when stored in gunny bags and exposed to natural infestation conditions. As protein intake is determined from food intake, it followed the same pattern as that of food intake. The casein diet led to the highest protein intake (18·28 g per rat) and it differed significantly from the remaining diets. Protein intake (8·72 g per rat) was markedly lower in diets having 75% infested grains as compared to diets containing uninfested grains (11·33 g per rat).

Gain in body weight of rats was highest on the casein diet (59·39 g per rat) and lowest (9·20 g per rat) on the diet having 75% insect-infested grains. The lower growth rate on infested grains may be attributed to deterioration in protein quality (Jood, 1990) as has also been observed in the case of sorghum, maize and pearl millet (Rajan *et al.*, 1975; Pant & Susheela, 1977; Kapoor & Kapoor, 1990).

FER was maximum on the casein diet (0·26). FER of the diet containing 75% infested grains was significantly ($p \leq 0{\cdot}05$) lower than that of the diet containing uninfested grains. Similarly, PER of grains showing 75% insect damage was significantly lower as compared to uninfested grains or the casein diet. A significant reduction in PER as a result of insect infestation shows that flour, besides being unpalatable, was also not conducive to body weight gain in rats, thereby showing poor utilization of proteins. Pant and Susheela (1977) also reported a decline in PER of insect-infested sorghum grains. However, the authors have not described the composition of the insect species involved and the extent of infestation.

Nitrogen consumption and absorption, digestibility, biological value (BV) and net protein utilization (NPU)

A significant reduction in nitrogen consumption in diets having 50% and 75% insect-infested grains was observed (Table 3). Similarly, there was a proportional decrease in nitrogen absorption with the increase in insect-infested grains in the diets. Rats absorbed the highest amount of nitrogen from the casein diet. Diets containing 50% and 75% insect-infested grains

Table 2. Food intake, protein intake and body weight gain of rats, and FER and PER[a] of insect-infested grains of wheat fed to rats for four weeks

Dietary group	Infestation level (%)	Food intake (g)	Protein intake (g)	Body weight gain (g)	FER	PER	Corrected[b] PER
Casein	0	228·45 ± 6·28	18·28 ± 2·20	59·39 ± 2·00	0·26 ± 0·03	3·24 ± 0·21	2·50
Wheat	0	141·46 ± 4·00	11·33 ± 1·49	17·95 ± 1·20	0·13 ± 0·01	1·59 ± 0·12	1·23
	25	137·41 ± 5·29	10·99 ± 2·11	15·85 ± 1·59	0·11 ± 0·02	1·44 ± 0·11	1·11
	50	132·58 ± 6·28	10·60 ± 1·60	13·13 ± 1·00	0·10 ± 0·01	1·24 ± 0·16	0·96
	75	109·00 ± 5·20	8·72 ± 1·00	9·20 ± 1·00	0·08 ± 0·00	1·06 ± 0·14	0·82
	SE (*m*)	6·52	1·25	1·65	0·01	0·22	
	CD ($p \leq 0·05$)	19·52	3·45	4·92	0·03	0·65	

[a]Values are means ± SD of eight rats in each group.
[b]Based on values of 2·5 as standard for casein.

Table 3. AD, TD, BV, NPU, UP and DMD values[a] of insect-infested grains of wheat fed to rats for five days

Dietary group	Infestation level (%)	Nitrogen consumed (mg/rat per day)	Nitrogen absorbed (mg/rat per day)	AD (%)	TD (%)	Nitrogen retained (mg/rat per day)	BV (%)	NPU (%)	UP (%)	DMD (%)
Casein	0	104·00 ± 8·85	95·27 ± 6·00	90·50 ± 3·90	91·61 ± 4·25	87·10 ± 4·00	91·42 ± 4·90	83·75 ± 4·21	67·00 ± 4·21	90·14 ± 3·88
Wheat	0	78·95 ± 5·52	69·92 ± 5·09	85·21 ± 2·41	88·56 ± 3·59	58·52 ± 3·92	83·70 ± 3·61	74·12 ± 3·00	8·75 ± 0·52	85·77 ± 2·88
	25	75·09 ± 5·42	64·49 ± 4·90	81·22 ± 4·62	85·88 ± 2·50	53·42 ± 4·02	82·83 ± 2·59	71·13 ± 2·90	8·11 ± 0·40	85·00 ± 2·37
	50	68·42 ± 2·49	55·42 ± 6·20	76·45 ± 3·62	81·00 ± 2·93	43·75 ± 2·98	78·94 ± 3·89	63·94 ± 2·12	7·03 ± 0·23	81·56 ± 4·32
	75	61·40 ± 6·20	45·28 ± 5·39	69·67 ± 3·01	73·75 ± 4·32	34·25 ± 4·32	73·43 ± 4·21	54·15 ± 3·25	5·66 ± 0·19	77·21 ± 2·58
	SE (*m*)	3·50	4·22	4·24	4·75	3·25	4·50	3·25	0·75	3·50
	CD ($p \leq 0·05$)	10·45	12·55	12·60	14·27	9·75	13·25	9·72	2·27	10·45

[a] Values are means ± SD of six rats in each group.

Table 4. NPR and PRE values[a] of insect-infested grains of wheat fed to rats for five days

Dietary group	Infestation level (%)	Weight gain (%)	Weight loss (%)	Protein consumed (g)	NPR	PRE
Casein	0	15·50 ± 2·10	2·35 ± 0·20	3·25 ± 0·11	5·49 ± 0·82	87·84 ± 8·32
Wheat	0	4·50 ± 0·21	2·35 ± 0·20	2·25 ± 0·10	3·04 ± 0·75	48·64 ± 2·52
	25	3·80 ± 0·10	2·35 ± 0·20	2·14 ± 0·09	2·87 ± 0·69	45·92 ± 2·00
	50	3·10 ± 0·11	2·35 ± 0·20	1·95 ± 0·08	2·74 ± 0·28	43·84 ± 1·52
	75	2·10 ± 0·31	2·35 ± 0·20	1·75 ± 0·09	2·54 ± 0·18	40·64 ± 2·12
	SE (*m*)	1·00		0·25	0·52	3·25
	CD ($p \leq 0{\cdot}05$)	3·00		0·72	1·50	9·72

[a]Values are means ± SD of six rats in each group.

showed significantly ($p \leq 0{\cdot}05$) poor nitrogen absorption.

Apparent digestibility (AD) of diets having uninfested grains (85·21%) did not differ significantly from the casein diet (90·50%) but declined sharply (69·67%) in the diet containing 75% insect-infested grains due to a portion of the proteins being eaten by the insects. True digestibility (TD) also showed a similar trend, as was observed for AD. One of the reasons for poor digestibility of infested grains may be a decrease in the digestible portion of proteins (eaten by insects) and accumulation of the undigestible portion. The other factors may be the cross-linking between proteins and other nutrients, such as sugars, oxidative products of lipids, aldehydes, etc., during infestation and storage, which have been reported to lower the digestibility (Cheftel, 1977; Saio, 1980). The antinutrients increasing during infestation may also decrease digestibility (Jood, 1990). Nitrogen retention was also significantly poor in rats fed on diets containing 50% and 75% insect-infested grains. Biological values of diets containing uninfested grains (83·70%) and casein (91·42%) were significantly higher than diets having 75% infested grains (73·42%). NPU and utilizable protein also showed a similar trend. The lower BV and NPU of infested grains may be due to loss, to a varying degree, of some of the essential amino acids. Oxidizing lipids can cause damage to arginine, phenylalanine, serine, methionine, tyrosine, threonine, lysine and cysteine (Karel, 1973). Dry matter digestibility of the casein diet was similar to the diet composed of uninfested grains. Diets having 75% infested grains showed significantly poor digestibility when compared to uninfested grains.

Net protein retention and protein retention efficiency

The values of NPR and PRE were significantly ($p \leq 0{\cdot}05$) higher in casein and uninfested grains than in infested grains (Table 4). Among the infestation levels, 75% gave the lowest NPR and PRE.

It may be inferred from the present studies that insect infestation causes deleterious effects on food intake, growth and nitrogen balance of rats, leading to poor utilization of protein.

REFERENCES

AOAC (1980). *Official Methods of Analysis* (13th edn). Association of Official Analytical Chemists, Washington, DC.

Atwal, A. S. (1976). *Agricultural Pests of India and South-East Asia.* Kalyani Publishers, Ludhiana, India.

Bender, A. E. & Doell, B. H. (1957). Biological evaluation of proteins, a new aspect. *Brit. J. Nutr.*, **11**, 140–8.

Chapman, D. C., Castillo, R. & Campbell, J. A. (1959). Evaluation of protein in foods. *Can. J. Biochem. Physiol.*, **37**, 679–85.

Cheftel, J. C. (1977). Chemical and nutritional modification of food proteins due to processing and storage. In *Food Proteins*, ed. J. R. Whitaker & S. T. Tannenbaum. AVI Publishing Co., Westport, CT, p. 401.

Chick, H., Hutchinson, J. C. D. & Jackson, H. M. (1935). The biological value of protein. VI. Further investigation of balance sheet method. *Biochem. J.*, **29**, 1702–11.

Daniel, V. A., Rajan, P., Sanjeevarayappa, K. V., Srinivasan, K. S. & Swaminathan, M. (1977). Effect of insect infestation on the chemical composition and protein efficiency ratio of the protein of Kaffir corn and green gram. *Indian J. Nutr. Dietet.*, **14**, 38–42.

Dass, S. (1977). Survey of insect pests in rural storage structures of Haryana and estimation of quantitative and qualitative losses caused by them. PhD thesis, Haryana Agricultural University, Hisar, India.

Girish, G. K., Kumar, A. & Jain, S. K. (1975). Part VI: Assessment of the quality loss in wheat damaged by *Trogoderma granarium* Everts during storage. *Bull. Grain Technol.*, **13**, 26–32.

Gupta, H. O., Lodha, M. L., Mehta, S. L., Rastogi, D. K. & Singh, J. (1979). Effect of amino acid(s) and pulse supplementation on nutritional quality of normal and modified opaque-2 maize. *J. Agric. Food Chem.*, **27**, 787–90.

Hira, C. K., Sadana, B. K., Mann, S. K., Kochhar, A., Kanwar, J. & Sidhu, H. (1988). Changes in chemical constituents of wheat stored in different storage structures. *Bull. Grain Technol.*, **26**, 109–16.

Jood, S. (1990). Studies on nutritional quality of wheat, maize and sorghum as affected by infestation of *Trogoderma granarium* and *Rhizopertha dominica.* PhD thesis, Haryana Agricultural University, Hisar, India.

Kapoor, R. & Kapoor, A. C. (1990). Biological evaluation of pearl millet protein: Effect of different treatments and storage. *Plant Food Human Nutr.*, **40**, 175–83.

Karel, M. (1973). Symposium on protein interaction in biosystem. Protein lipid interactions. *J. Food Sci.*, **38**, 756–63.

Pant, K. C. & Susheela, T. P. (1977). Effect of storage and insect infestation on the chemical composition and nutritive value of grain sorghums. *J. Sci. Food Agric.*, **28**, 963–70.

Pingale, S. V. & Girish, G. K. (1967). Effect of humidity on the development of storage insect pests. *Bull. Grain Technol.*, **5**, 101–8.

Platt, B. S., Miller, D. S. & Payne, P. R. (1961). Protein values of human foods. In *Recent Advances in Human Nutrition*, ed. J. F. Brock. Little, Brown and Co., Boston, pp. 351–8.

Pushpamma, P. & Reddy, M. U. (1979). Physicochemical changes in rice and jowar stored in different agroclimatic regions of Andhra Pradesh, *Bull. Grain Technol.*, **17**, 97–108.

Rajan, P., Daniel, V. A., Padamarani, R. & Swaminathan, M. (1975). Effect of insect infestation on the protein efficiency ratio of the maize and cowpea. *Indian J. Nutr. Dietet.*, **12**, 354–7.

Saio, K. (1980). Nutritive losses in storage and processing of legumes. In *Nutrition and Food Science*, ed. W. Santos, M. Lopes, J. J. Barbosa, D. Charles & J. C. Valente. Plenum Press, New York, pp. 421–49.

Sharma, S. S., Thapar, V. K. & Simwat, G. S. (1979). Biochemical losses in stored wheat due to infestation of some stored grain insect pests. *Bull. Grain Technol.*, **17**, 144–7.

Snedecor, G. W. & Cochran, W. G. (1968). *Statistical Methods.* Oxford IBH Publishing Co., Calcutta, India.

Swaminathan, M. (1977). Effect of insect infestation on weight loss, hygienic condition, acceptability and nutritive value of food grains. *Indian J. Nutr. Dietet.*, **14**, 205–16.

Viljoen, J. H. (1990). The occurrence of *Trogoderma* (Coleoptera: Dermestidae) and related species in Southern Africa with special reference to *T. granarium* and its potential to become established. *J. Stored Prod. Res.*, **26**, 43–51.