Biological Evaluation of Protein Quality of Maize As Affected by Insect Infestation

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

INTRODUCTION

Maize is a valuable and less expensive source of protein, carbohydrates, B vitamins, and some minerals. Stores of maize grains in rural areas are attacked by a large number of insect pests in Asia and Africa (Atwal, 1976; Salunkhe et al., 1985). Among these, *Trogoderma granarium* Everts and *Rhizopertha dominica* Fabricius are most serious; they coexist and are of wider occurrence in stored cereals in tropical and subtropical regions of Asia and Africa (Atwal, 1976; Das, 1977; 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). On the other hand, insect infestation was reported to increase total protein, non-protein nitrogen, and uric acid in infested grains (Swaminathan, 1977; Hira et al., 1988), but the true protein content of such grains has been reported to decrease (Jood, 1990; Girish et al., 1975; Pushpamma and Reddy, 1979). Hence, the real nutritional value of protein is not reflected in chemical analysis. Moreover, a number of factors such as the presence of toxic nitrogenous substances, insect excreta, and body fragments, and proteinase inhibitors are not accounted for in chemical analysis. In such a situation biological evaluation of protein quality is most desirable. This paper reports the effect of different levels of grain infestation (25, 50, and 75%) caused by mixed population of *T. granarium* and *R. dominica* on protein quality through rat growth and nitrogen balance studies.

MATERIALS AND METHODS

Mass cultures of two insect species (*T. granarium* and *R. dominica*) were maintained in the ambient laboratory temperature (28–39 °C) and relative humidity (60–90%) conditions. The grains of a commonly consumed variety of maize (Composite Vijay), apparently free from insect infestations, were procured and further subjected to aluminum phosphide fumigation to eliminate any untraced insect population. After fumigation, such grains were put in 36 glass jars (20 × 15 cm), each containing 1.5 kg of grains. The jars were covered with muslin cloth held in place by elastic bands and placed in the laboratory for 10 days for conditioning of grains. On the 10th day, the moisture level of grains ranged from 10 to 11%, which is favorable for multiplication of both insect species (Pingale and 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 the second set, 60 adults of *R. dominica* were released, while in the third set a mixed population of both species (30 larvae of *T. granarium* plus 30 adults of *R. dominica*) was released to achieve three infestation levels in three replications. In addition to this, in each set, controls (jars without insects) were also kept simultaneously to compare the results with uninfested grains. To achieve desired infestation levels, grain samples (500 grains/jar) were inspected twice a week after the release of insects; grains that showed signs of insect damage were considered to be infested. Infestation level (percent) was calculated by dividing the number of insect-damaged grains by the number of total grains inspected and then mutliplying by 100. 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 aluminum phosphide fumigation to halt further insect activity 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 infestation due to initial exposure to aluminum phosphide. After fumigation at each infestation level, grains were cleaned by passing through a 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 mill to pass through a 60-mesh sieve and then stored in airtight polyethylene bottles for further chemical analysis.

Test Animals. Young white male albino Wistar rats weighing 25 ± 5 g were obtained from the Disease and Germ Free Small Animal House, C. C. S. Haryana Agricultural University, Hisar. 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. The composition of the diets which contained uninfested and infested maize flour so as to provide protein at 8% level is given in Table I. One group of the rats was fed a synthetic diet containing casein as the source of protein at 8% level. This group served as 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 1 week at a time and

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kept in the refrigerator. All of the diets were analyzed for their moisture and protein content (AOAC, 1980).

Growth Experiment. The protein efficiency ratio (PER) was determined according to the method of Chapman et al. (1959). The rats were initially weighed and then weighed on every alternate day. The weighed diet was daily given, 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 4 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 using the formulas

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

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

Nitrogen Balance Studies. For nitrogen balance studies, adult male Wistar rats were housed individually in polypropylene metabolic cages. One group of rats was fed a protein-free diet and another a casein diet. Four groups of rats were fed test diets (containing flours of grains showing 0, 25, 50, and 75% insect infestations). The experiment was conducted for 9 days, which included an initial conditioning period of 4 days. During the last 5 days, urine and feces 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 feces was determined according to the micro-Kjeldahl 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), protein retention efficiency (PRE) (Bender and Doell, 1957), dry matter digestibility (DMD), and utilizable proteins (UP) (Gupta et al., 1979) by employing the formulas

$$\text{AD} = (\text{NI} - \text{NF1} \times 100)/\text{NI}$$

$$\text{TD} = (\text{NI} - (\text{NF1} - \text{NF2}) \times 100)/\text{NI}$$

$$\text{BV} = (\text{NI} - (\text{NF1} - \text{NF2}) - (\text{NU1} - \text{NU2}) \times 100)/(\text{NI} - (\text{NF1} - \text{NF2}))$$

NI = N intake of animals fed test diet

NF1 = N excreted in feces of animals fed test diet

NF2 = N excreted in feces of animals fed protein-free diet

NU1 = N excreted in urine of animals fed test diet

NU2 = N excreted in urine of animals fed protein-free diet

$$\text{NPU} = (\text{BV} \times \text{TD})/100$$

NPR = (gain in weight of test animals + loss in weight of control animals)/weight of protein consumed by test animals

$$\text{PRE} = \text{NPR} \times 16$$

$$\text{UP} = (\text{NPU} \times \text{N}\ (\%\ \text{of dry matter}) \times 6.25)/100$$

$$\text{DMD} = (\text{food consumed} - \text{fecal weight} \times 100)/\text{food consumed}$$

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 and Cochran, 1968).

Table I. Composition[a] of the Experimental Diets

		maize			
			infested		
ingredient	casein	uninfested	25%	50%	75%
casein	100				
flour		708.0	735.3	764.8	807.3
sucrose	50	50	50	50	50
groundnut oil	100	76.5	75.9	75.5	74.8
mineral mixture	40	40	40	40	40
vitamin mixture	10	10	10	10	10
cellulose	50				
DL-methionine	3.0				
choline bitartrate	2.0	2.0	2.0	2.0	2.0
cornstarch	645.0	113.5	86.8	57.7	15.9

[a] Grams per kilogram of diet.

RESULTS AND DISCUSSION

Food Efficiency Ratio and Protein Efficiency Ratio. Food intake of the casein diet (control) was the highest (228.45 g/rat), and it differed significantly ($P < 0.05$) from that of other diets (Table II). Consumption of diets containing flour of 75% infested maize 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. As protein intake is determined from food intake, it followed the same pattern as that of food intake. The casein diet registered the highest protein intake (18.28 g/rat), which differed significantly from that of the remaining diets. Protein intake (5.73 g/rat) was markedly lower in diets having 75% infested grains as compared to diets containing uninfested grains (10.22 g/rat).

Gain in body weight of rats was highest for the casein diet (59.39 g/rat) and lowest (6.02 g/rat) for the diet having 75% insect-infested grains; this may be attributed to deterioration in protein quality (Rajan et al., 1975; Jood and Kapoor, 1992) as was also observed in the case of pearl millet (Kapoor and Kapoor, 1990).

FER was maximum for the casein diet (0.26). The FER of the diet containing 75% infested grains was significantly ($P < 0.05$) lower than that of the diet containing uninfested grains. Similarly, the PER of grains showing 75% insect damage was markedly lower as compared to that of uninfested grains and casein diet. A significant reduction in PER as a result of insect infestation indicates that flour, besides being unpalatable, was also not conducive for body weight gain in rats, thereby showing poor utilization of proteins. In earlier studies (Swaminathan, 1977) also, maize insect-infested grains fed to rats resulted in substantial reduction in PER values.

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 III). 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 showed significantly ($P < 0.05$) poorer nitrogen absorption.

The apparent digestibility (AD) of the diet having uninfested grains (84.20%) did not differ significantly from that of the casein diet (90.50%) but declined sharply (69.09%) in the diet containing 75% insect-infested grains due to the amount of protein eaten by insects. True

Table II. Food Intake, Protein Intake, and Body Weight Gain of Rats and FER and PER[a] of Insect-Infested Grains of Maize Fed to Rats for 4 Weeks

dietary group	infestation level, %	food intake, g	protein intake, g	body wt gain, g	FER	PER	corrected PER[b]
casein	0	228.45 ± 6.28	18.28 ± 2.20	59.39 ± 2.00	0.26 ± 0.03	3.24 ± 0.21	2.50
maize	0	127.54 ± 8.20	10.22 ± 1.58	15.29 ± 2.26	0.12 ± 0.01	1.50 ± 0.09	1.16
	25	119.80 ± 8.60	9.59 ± 2.00	13.09 ± 2.00	0.11 ± 0.01	1.37 ± 0.08	1.06
	50	100.39 ± 5.32	8.02 ± 1.21	9.22 ± 1.15	0.09 ± 0.01	1.15 ± 0.10	0.89
	75	71.58 ± 4.49	5.73 ± 0.99	6.02 ± 1.21	0.08 ± 0.00	1.05 ± 0.09	0.81
SE (*m*)		6.52	1.25	1.65	0.01	0.22	
CD ($P < 0.05$)		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 casein.

Table III. AD, TD, BV, NPU, and DMD Values[a] of Insect-Infested Grains of Maize Fed to Rats for 5 Days

dietary group	infestation level, %	N consumed, mg/day per rat	N absorbed, mg/day per rat	AD, %	TD, %	N retained, mg/day per rat	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 ± 3.81
maize	0	71.36 ± 3.42	62.61 ± 5.20	84.20 ± 3.00	87.74 ± 3.89	47.25 ± 2.19	75.47 ± 5.20	66.22 ± 4.90	7.48 ± 0.53	82.27 ± 3.51
	25	68.16 ± 2.19	57.40 ± 4.32	80.12 ± 4.19	84.21 ± 4.21	41.21 ± 2.20	71.79 ± 5.00	60.45 ± 4.00	6.58 ± 0.69	81.32 ± 3.51
	50	61.44 ± 3.92	40.10 ± 3.90	75.24 ± 3.50	79.92 ± 3.50	32.26 ± 3.19	65.70 ± 4.19	52.51 ± 2.79	5.49 ± 0.32	77.92 ± 3.27
	75	55.04 ± 1.99	40.39 ± 3.92	69.09 ± 4.92	73.38 ± 3.92	24.00 ± 3.00	59.42 ± 3.00	43.61 ± 3.00	4.32 ± 0.39	73.72 ± 2.92
SE (*m*)		3.50	4.22	4.24	4.75	3.25	4.50	3.25	0.75	3.50
CD ($P < 0.05$)		10.45	12.53	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 IV. NPR and PRE Values[a] of Insect-Infested Grains of Maize Fed to Rats for 5 Days

dietary group	infestation level, %	wt gain, %	wt 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
maize	0	3.95 ± 0.42	2.35 ± 0.20	2.23 ± 0.11	2.83 ± 0.15	45.28 ± 3.00
	25	3.18 ± 0.89	2.35 ± 0.20	2.13 ± 0.10	2.60 ± 0.12	41.60 ± 1.59
	50	2.41 ± 0.30	2.35 ± 0.20	1.92 ± 0.13	2.48 ± 0.32	39.68 ± 1.28
	75	1.75 ± 0.29	2.35 ± 0.20	1.72 ± 0.08	2.38 ± 0.13	38.08 ± 2.58
SE (*m*)		1.00		0.25	0.52	3.25
CD ($P < 0.05$)		3.00		0.72	1.50	9.72

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

digestibility (TD) also showed a similar trend. One of the reasons for poor digestibility of infested grains may be a decrease in the digestible portion of protein eaten by insects and accumulation of the undigestible portion. Other factors may be the cross-linking between protein and other nutrients such as sugar, oxidative products of lipids, and aldehyde during infestation and storage, which have been reported to lower the digestibility (Cheftal, 1977; Sai, 1980). Antinutrients such as polyphenol and phytic acid increased during infestation and may also decrease digestibility (Jood, 1990); they are known to deteriorate the nutrient quality of legumes and cereals (Reddy et al., 1982). Nitrogen retention was also significantly poorer in rats fed diets containing 50 and 75% insect-infested grains. Biological values of diets containing uninfested grains (75.47%) and casein (91.42%) were significantly higher than those of the diet having 75% infested grains (59.42%). NPU and utilizable protein also showed a similar trend. The lower BV and NPU values of infested grains may be due to varying degrees of losses of some essential amino acid. Oxidizing lipids can cause damage to arginine, phenylalanine, serine, methionine, tyrosine, and cysteine (Karel, 1973). The dry matter digestibility of the casein diet was on par with the diet consumed of uninfested grains. The diet having 75% infested grains showed substantially poorer digestibility when compared to that of uninfested grains.

Net Protein Retention and Protein Retention Efficiency. The NPR and PRE values were significantly higher for casein than for infested grains (Table IV). Among infestation levels, 75% had the lowest NPR and PRE values. However, a 25% level of infestation did not cause any appreciable change in protein quality. In earlier studies (Belvady and Deosthale, 1980) also, adverse effects on protein quality of sorghum grain infested to the extent of 30% were not observed.

It may be inferred from the present studies that insect infestations (25–75%) cause deleterious effects on food intake, growth, and nitrogen balance of rats, leading to poor utilization of protein and thus overall poor quality of proteins.

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