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THE EFFECT OF CONTAMINATION OF PEANUT MEAL WITH *ASPERGILLUS FLAVUS* ON PROTEIN QUALITY AS MEASURED CHEMICAL AND IN BIOASSAYS ON CHICKENS AND DUCKLINGS

H T Ostrowski-Meissner[1], Ir Winarso Siswohardjono, Dadang Suherman and Idris Barchia

Balai Penelitian Ternak, Project for Animal Research and Development, (CSIRO-Australia), P O Box 123, Bogor, Indonesia

The protein quality of peanut (PNM) either aflatoxin-free or contaminated with aflatoxins was evaluated using either chemical assays as chemical score and predicted discriminant computed protein efficiency ratio (DC PER) or using various bioassays such as protein efficiency ratio (PER), net protein utilisation (NPU), total protein efficiency (TPE), plasma both total (PTAA) and esential (PEAA) amino acids, and nitrogen retention with both chickens and ducks being the test animals. When ducklings were used in bioassays as test animals because their high sensitivity to aflatoxins, a significant reduction in the bioassay results occurred when contaminated PNM was tested compared to the aflatoxin-free PNM. Bioassay on chickens and chemical assays were proved to be insensitive in predicting an improvement of utilisation of dietary proteins by ducks fed PNM contaminated with aflatoxins. In the humid tropics where most of stored foods are infested with *Aspergillus flavus* a reduction in the utilisation of protein from foods contaminated with this fungus may be expected when fed to organisms sensitive to aflatoxins. Such a reduction may not be detected if chemical assays or bioassays with the use of test animals of low sensitivity to aflatoxins are used.

Key Words: Protein quality, aflatoxins, peanut meal, chemical assessment, bioassay, ducks, chicken

Bainton and Jones (1977) reported that a high proportion of feedstuffs surveyed in various countries in the tropics, particularly in South East Asia, were contaminated with aflatoxins, exceeding the level of 50 µg aflatoxin B_1 equivalent/kg established by the FAO (Giddey et al 1977) as acceptable for animals. Storage of improperly dried crops is a major cause of infestations of foods and feeds with the fungus *Aspergillus flavus* with produces aflatoxins reported as potent hepatocarcinogens (Heathcote and Hibbert 1977) and the toxin responsible for a decline in the productive performance of the farms animal species sensitive to mycotoxins (Muller et al 1970, Ostrowski-Meissner 1981a). There are indications that the values describing protein quality of feeds contaminated with aflatoxins may differ, depending on both the assay from which results were derived and test animals used in bioassat (Ostrowski-Meissner 1981b, 1982). Thus there is a need for a reliable, timely and inexpensive rapid assay which could be used to evaluate protein quality in feeds infected with *Aspergillus flavus*. This seems to be of particular importance to humid tropical regions where most stored feeds are infected with fungi (Bainton and Jones 1977).

[1]*Present address: CSIRO, Division of Animal Production, Prospect, P. O. Box 239, Blacktown, NSW, 2148, Australia*

The concentrations of amino acids is commonly used in evaluating the protein quality of feeds and a number of measurements derived from amino acid profiles in protein have been adopted as criterions of quality (Mitchell and Block 1946; Hansen and Eggum 1973; Jewell et al 1980). The problem arises however, that the results of protein quality of feeds infected with *Aspergillus flavus*, derived from chemical analyses may differ from the results of the quality determined in bio-assays with the use of various types of laboratory animals. This is due to possible different sensitivity of the experimental animals to sub-lethal concentrations of aflatoxins in test feeds. This paper describes changes in results of the protein quality of the peanut (food/feed popularily-used not only in the tropics) due to contamination with the fungus *Aspergillus flavus*. Paper also desmonstrates the differences in protein quality results which have been derived from either amino acid composition or from standard bio-assays on two poultry species (chickens and ducks) known to have different sensitivity to aflatoxins (Muller et al 1979, Ostrowski-Meissner 1981a,b; 1982).

Materials and Methods

Peanut meal: A peanut crop grown in East Java and free of aflatoxins, was dried in the sun for 10 days after harvest and then placed in a forced draught dryer at a temperature of 65°C until 92% dry matter (DM). The peanuts were manually dehulled and oil extracted first in a press and then in a column chloroform extractor. The peanut cake was ground and passed through a 0.5 mm screen and then divided into two portions. One portion was contaminated with aflatoxins (AF) by mixing peanut meal free of AF (PNM_0) with chloroform extract containing aflatoxins (1,53 mg aflatoxin B_1 equivalent/ml) extracted from a culture of *Aspergillus flavus* grown on grain rice according to West et al (1973). After contamination peanut meal contained 373 µg aflatoxins B_1 equivalent/kg (PNM_{AF}). The second portion (PNM_0) was handled identically as PNM_{AF} except that chloroform without aflatoxins was used during a mixing process.

Diets: Except for PNM_{AF}, all dietary ingredients used in this study as casein used as a reference protein in the control diet were free of aflatoxins. Chemical composition of protein sources used is given in Table 1. Casein or peanut meal were incorporated in the test diets (Table 2) at the level providing 12% crude protein (268 g PNM/kg diet). This resulted in levels of 0 or 100 µg aflatoxin B_1 equivalent/kg diet when PNM_0 or PNM_{AF} were used respectively. An additional level of 50 µg aflatoxin/kg was achieved by mixing PNM_0 and PNM_{AF} in proportions 1:1 prior to inclusion in the test diet.

Birds: One-day-old Alabio ducklings and White Leghorn chickens were housed in cages with raised wire floors. At seven or fourteen days of age, they were weighed and selected for bioassays; usually 10 to 15 g separated the lightest and heaviest selected bird. The birds were then distributed to cages singly and in random order. Experimental diets were fed ad libitum to three groups of four birds for 14 days. Groups were weighed on the 7th and 14th days of the assay and food consumption recorded. Constant lighting was provided by 150 W lamps hung 25 cm above each cage when the

Table 1:

Chemical composition of the peanut meal (PNM_0) and casein both free of aflatoxins (AF) and peanut meal infected with AF (PNM_{AF}) used in protein quality assays

Constituent	PNM	PNM_{AF}	Casein
Proximate analyses:			
Dry matter (%)	88.7	88.1	90.8
Crude protein (N x 6.25)(% DM)	44.8	45.6	79.8
Ether extract (% DM)	20.4	23.0	0.5
Crude ash (% DM)	5.5	5.4	3.7
True Metabolizable Energy (MJ/kg)	13.8	14.1	16.9
Amino Acids (g/16 g N):			
Lysine	3.22	3.63	7.51
Methionine & cystine	2.17	1.65	2.96
Threonine	3.01	3.13	3.43
Isoleucine	4.51	4.40	5.01
Leucine	7.58	7.68	9.20
Valine	5.80	5.56	5.42
Phenylalanine	5.44	6.14	4.86
Tyrosine	3.41	4.58	5.12
Tryptophan	1.11	1.14	1.21
Aspartic acid	8.64	10.09	6.41
Proline	4.5	5.35	11.82
Cystine	1.41	0.72	0.15
Arginine	10.76	10.53	3.41
Glycine & serine	8.70	6.59	7.56
Histidine	2.11	2.75	2.20
Glycine	5.16	4.14	1.84

Table 1:

The composition of the basal diets (g/kg) used in the evaluation of protein quality using bioassays on ducklings and chickens

Ingredient	N-free diet	PER & NPU assays	TPE assays	Preliminary diets for	
				PER & NPU assay	TPE assay
Corn starch	464	335	-	-	-
Corn	-	-	661.8	237	629
Peanut meal	-	268	268	-	-
Soybean meal	-	-	-	235	290
Dried yeast	-	-	8.2	50	-
Leaf meal	-	-	4	-	-
Fish meal	-	-	-	-	50
Corn bran	-	-	-	100	-
Pollard	-	-	-	302	-
Meat & bone meal	-	-	-	30	-
Corn gluten	-	-	-	9	-
Sugar	464	335	-	-	0
Premix	30[1]	30[1]	30[1]	30[1]	30[2]
DL-methionine	0.6	0.6	0.6	0.3	1
Choline chloride	1.4	1.4	1.4	1.7	-
Corn oil	40	40	26	5	-
TOTAL	1000	1000	1000	1000	1000
Crude protein (N x 6.25)	0.0	120	185	220	220
TME (MJ/kg)	16.1	15.9	15.2	14.4	14.6

[1] Providing (mg): 15 calcium panthothenate, 6 riboflavin, 3 thiamin hydrochloride, 4 pyridoxine hydrochloride, 0.2 biotin, 40 nicotinic acid, 1.5 pteroylmonoglutamic acid, 0.04 cholcalciferol, 10 α-tocopheryl acetate, 5 menaphthone, 5 retinol (as Rovimix A_{235}); and 20 µg cyanocobalamin, 20 g $CaHPO_4$ x $2H_2$), 2.7 g $MgSO_4.H_2$), 670 mg $FeSO_4$ + 7 H_2O, 270 mg $MnSO_4.4H_2O$, 130 mg $ZnSO_4.7H_2$), 16 mg $CuSO_4.5H_2O$, 37 mg KI. Mineral premix was mixed with vitamins at the time of preparation of the diet.

[2] As [1] but providing extra 0.50 mg niacin.

birds were seven-days-old. This gave an ambient temperature of approximately 34°C; the lamps were lifted gradually up to 70 cm above each cage when birds reached the age of 21-days-old. This resulted in an ambient temperature of approximately 25°C.

Bioassays: Protein Efficiency Ratio (PER) and Net Protein Utilisation (NPU) were determined using the principle of the method for rats as described by Bender and Miller (1953). Seven-day-old ducklings or chickens were used for each test protein sample and also for the casein control and N-free groups. The weights of birds for the PER determination were taken

on the 14th day of feeding trial. Birds were fasted for 12 hours then re-weighed and killed with ether, dried to constant weight in an oven at 85° C and the moisture content calculated. One replicate of ducklings and chickens from each treatment was ground, analysed for nitrogen on a fat-free basis and the water/nitrogen ratio calculated for NPU. This ratio was then used to estimate the percentage of nitrogen in that treatment's remaining replicates. It has been demonstrated by Summers and Fisher (1961) that such a ratio is constant both within and between treatments under uniform experimental conditions.

Total Protein Efficiency (TPE) was determined using the principle of the method described by Woodham (1968) on 14-days-old birds with the test diets resembling practical rations and containing 18.5% crude protein (CP) being assessed during 14 days feeding period.

Prior to the end of the TPE assay during three consecutive 24 hour periods birds were given experimental diets containing chromic oxide dough (2 g/kg). A total excreta collection was made on a group replicate basis and the gross balance of dietary nitrogen was determined. At the end of the nitrogen balance study, an ileal digestion of amino acids was determined using the principle described by Varnish and Carpenter et al (1975).

At the time of killing, blood was taken from the jugular vein of birds fed ad libitum, in heparinized tubes for plasma amino acids determination, then the livers were dissected, weighed and a semi-quantitative evaluation of gross appearance of livers was made.

True metabolisable energy (TME) in the feeds was determined according to Sibbald (1976) on adult (8-months-old) Single Comb White Leghorn roosters with the use of 24 h excreta collection.

Chemical assays: The chemical score was calculated according to the principle outlined by Michell and Block (1946) with the amino acid requirement of 3-week-old broiler (NRC 1977) taken as protein standard for both chickens and ducks. The discriminant computed protein efficiency ratio(DC-PER) was determined by the use of a technique by Jewell et al (1980) with the FAO/WHO (1973) reference essential amino acid pattern being applied as a standard.

Analyses: Aflatoxins were determined by the method of Pons et al (1972 The levels of aflatoxins in both chloroform extracts and feeds were expressed as equivalent of aflatoxin B_1 based on the relative toxicity of aflatoxins B_1, B_2, G_1 and G_2 for ducklings (Carnaghan et al 1963).

The nitrogen in test protein, excreta and defatted carcasses was determined using a Technicon Auto Analyser II, method No. 334-74 A/A. Other approximate analyses were conducted according to AOAC (1975) methods. Amino acid contents were determined in acid hyrolysates (6 N HCl) on a Beckman Amino acid Analyser using the procedure as supplied by the manufacturer. Tryptophan was determined after hydrolysates with barium hydroxide using the procedure of Matheson (1974).

A Hewlett Packard Model 9831A mini computer was used for chemical assessment of test proteins from the amino acid data.

Results

With an increase in the aflatoxin (AF) concentration from 0 to 50 µg/kg the results of the assays on ducks which are based on overall body weight gain (PER and TPE) as well as ileal digestion of amino acids were not significantly affected. There was however a significant reduction in those measurements reflecting nitrogen deposition (NPU, nitrogen retention) and concentration of amino acids in plasma (Table 3). With ducklings being used as test animals, and increase in dietaty aflatoxin concentration from 0 to 100 µg aflatoxin B_1 equivalent per kg diet resulted in a significant lowering of the all bioassay results. In contrast no significant effect on the results of bioassays on chickens was observed at either level of dietary AF.

With an elevation in dietary aflatoxin level the weight of livers of ducks significantly increased and a higher incidence of liver damage was observed (Table 4). Similar changes were not observed in chickens.

Chemical score (CS) and predicted computed PER (DC-PER) derived from the results on amino acid composition were not affected by the presence of aflatoxins in the ration used (see Table 3).

There were no significant difference in the results of the bioassays between ducks and chickens when PNM_0-aflatoxin free was used. However, as the aflatoxin concentrations in rations increased, the difference between the results of bioassays on ducks and chickens became greater (see Tables 3 and 4).

Discussion

The results confirm that ducks are more sensitive to AF as compared to chickens (Muller et al 1970, Ostrowski-Meissner 1981a) and also that these two animal species used in protein quality biossays have different patterns of growth and other measurements reflecting utilisation of proteins from feed contaminated with aflatoxins. The utilisation of protein, from feeds contaminated with aflatoxins, by ducklings was already retarded at the level 50 µg/kg, i.e. at the level quoted by Giddey et al (1977) as a concentration acceptable by the FAO. According to surveys conducted by Bainton and Jones (1977) and Shotwell et al (1976) a large proportion of feeds and foods consumed in countries located in South East Asia, Africa, South America are contaminated with aflatoxins, above the level of 50 µg/kg which significantly retarded protein metabolism in ducks fed PNM_{AF}. During the survey conducted in Indonesia (Hetzel and Sutikno 1979) AF levels above 100 µg/kg feedstuffs were recorded. Countries importing food and feedstuffs from tropical regions have established tolerable aflatoxin levels below 50 µg/kg (Krough 1977) Therefore, the problem of dietary aflatoxin interfering with the normal process of protein metabolism may be prominant in those tropical countries which are exporting foods and feeds with low aflatoxin levels and those foods rejected from export due to their high contamination with AF and lack of regulations as to the tolerable AF levels are utilised locally. In the tropics particularly in South East Asian countries like Indonesia and Philippines where intensification of duck production is envisaged and where contamination of dried feeds with fungus *Aspergillus flavus*

Table 3:

The effects of contamination of the peanut meal with aflatoxins on the results of various bioassays conducted on chickens (C) and ducklings (D) and with the use of chemical assays

	Test animal	Peanut meal (aflatoxin level µg/kg diet) Aflatoxin-free (0)	Aflatoxin-contaminated (50)	(100)	Pooled SE of mean
BIOASSAY[1]:					
Protein Efficiency Ratio[2] (PER)	C	1.97a	2.00a	2.01a	0.042
	D	2.09a)NS	1.93a)NS	1.68b)**	
Net Protein Utilization[3] (NPU)	C	31.2a	31.8a	32.0a	0.903
	D	32.4a)NS	28.7b)*	20.5c)**	
Total Protein Efficiency[4] (TPE)	C	1.56a	1.59a	1.53a	0.117
	D	1.63a)NS	1.55a)NS	1.08b)*	
Ileal digestibility of amino acids[5] (IDAA)	C	0.82a	0.84a	0.80a	0.052
	D	0.83a)NS	0.79a)NS	0.61b)*	
Plasma amino acids (µmol/100 ml)[6]:					
Lysine (PL)	C	7.62a	7.68a	7.76a	0.241
	D	7.85a)NS	6.9ab)NS	6.02a)*	
Total amino acids (PTAA)	C	241a	244a	240a	10.5
	D	257a)NS	221b)NS	204b)*	
Essential amino acids (PEAA)	C	96.4a	95.3a	96.9a	3.55
	D	98.9a)NS	84.4b)*	70.7c)**	
Nitrogen Retention (g/g N ingested)	C	0.59a	0.63a	0.60a	
	D	0.64a)NS	0.53b)*	0.41c)**	
CHEMICAL ASSAY[7]:					
Chemical Score (CS)[8]		49.8	50.1	50.0	ND[9]
Predicted PER (DC-PER)[10]		1.74	1.76	1.76	ND

[1] Each value represents mean of three determinations, each on four animals; values in the same line which do not share a common letter are significantly different ($P \leq 0.05$) according to the Tukey procedure. Significance displayed in the columns are referring to the differences between chickens and ducks at certain aflatoxin levels; NS = not significant; * = significance at $P < 0.05$ level; ** = significances at $P < 0.01$ level.

[2] Liveweight gain (g)/protein consumed (g) with the test (12% CP) diet,

[3] (body N with test protein) -(body N with protein-free diet)/total N intake.

[4] Liveweight gain (g)/protein consumed with the test practical (18,5% CP) diet (g),

[5] Apparent digestibility coeficient = 1-(weight of amino acids per unit weight chromic oxide in ileal digesta from birds fed test diet/amino acids per unit weight chromic oxide in the test diet)

[6] Blood collected from birds fed ad libitum and killed between 8:00 and 10:00 am.

[7] Based on one amino acid analysis.

[8] In calculations the amino acid requirements of 3-week-old broilers according to the NRC (1977) was taken as the protein standard.

[9] Not determined.

[10] In discriminant computed Protein Efficiency Ratio model (Jewell et al 1980) the standard reference essential amino acid pattern as proposed by FAO/WHO(1973)was applied.

Table 4:

The effect of contamination of the peanut meal with aflatoxins on the post-mortem examination of livers in chickens (C) and ducks (d)[1]

Measurement		Peanut meal (aflatoxin level µg/kg diet)[2] Aflatoxin-free	Aflatoxin-contaminated		Pooled SE of mean
Liverweight (g)	C	4.5a)NS	4.6a)*	4.5a)**	0.144
	D	4.8c	5.4b	5.9a	
Pathology score[3]	C	1.0a)NS	1.0a)NS	1.0a)**	0.181
	D	1.0c	1.4b	2.2a	
Histology score[4]	C	1.0a)NS	1.0a)*	1.0a)**	0.179
	D	1.0c	1.6b	2.5a	

[1] Each value represents the mean of 12 livers collected from three groups each of 4 animals

[2] Values in the same row with unlike superscript are significantly different ($P < 0.05$) according to the Tukey's procedure. Significance displayed in the columns are referring to the differences between ducks and chickens at certain aflatoxin levels; NS = no significant; * = significance at $P < 0.05$; ** = significance at $P < 0.01$

[3] Pathology score : 1 = normal (mahogany), 2 = pale, 3 = green

[4] Histological score: 1, no lesion; 2, mild lesion - early hyperplasia of bile duct without necrosis; 3, medium lesion - bile duct hyperplasia affecting less than 50% of liver lobules and necrosis; 4, severe lesion-bile duct hyperplasia affecting more than 50% of liver lobules and necrosis

cannot be prevented and where proper storage of feeds is difficult, optimum duck production under an intensive husbandry system may not be achieved due to development of pathological manifestations that impair utilisation of dietary proteins. Therefore, chemical assays based on the amino acid composition of test protein may not be a true prediction of the quality of protein contaminated with aflatoxins when fed to organism sensitive to aflatoxins.

Smith et al (1981) reported that the detrimental effects of aflatoxins on animals can be markedly influenced by protein nutrition, and explained this by the fact may mycotoxins usually increase the protein requirements of animals. While this could be true for animals like ducks which are sensitive to aflatoxins, Rajion and Farrell (1976) reported no effect of AF on nitrogen balance in chickens. The significant reduction in nitrogen retention, NPU and plasma amino acid concentration observed in ducks fed PNM_{AF} may indicate an impairment in absorption-deposition-utilisation of protein from food contaminated with AF.

Carnaghan et al (1963), Tung et al (1972) and Bryden et al (1979) demonstrated a reduction in liver RNA concentration in chickens fed a diet with a high aflatoxin level (2 mg/kg), a fact suggesting that aflatoxin impairs protein synthesis. The present study indicates that an impairment

and a reduction in amino acid absorption occur already at aflatoxin concentration 50 and 100 µg/kg while chickens are not at all affected by such toxin levels. This may be one possible explanations for differences in the utilisation of protein from peanut meal as determined in bioassay on chickens and on ducks. Another explanation may be liver damage observed in ducks consuming diets contaminated with aflatoxins while livers in chickens were not affected.

The liver lesions for ducks fed a diet containing 50 and 100 µg aflatoxin B_1 equivalent per kg consisted mainly of bile duct hyperplasia and degenerative changes in liver parenchymal cells ranging from cloudy swelling to severe necrosis. Bile duct hyperplasia is recognized as the most characteristic and easy identifiable result of afatoxin poisoning although it is not specitic to aflatoxins (Heathcote and Hibbert 1977).

In conclusion it apperars that in the humid tropics where many stored foods are infested with *Aspergillus flavus* producing toxins-aflatoxins, the utilisation of protein from infected foods may be substantially decreased when fed to farm animals, sensitive to these toxins. Such a decrease may not be predicted either by the chemical assays (chemical score or DC-PER), nor when animal species with low susceptibility to aflatoxins is used in a bioassay.

When contamination of foods with aflatoxins is envisaged, as is common in humid tropics, the nutritive quality of food protein should be assessed with the use of a bioassay in which metabolism is used as a basis of the quality assessment and again using a test organism for consumption of which contaminated food is envisaged.

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