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Genetic Variation of Physiological Response to Aflatoxin in *Gallus domesticus*

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Summary. A pedigreed, commercial broiler population of 31 sire families was administered dietary aflatoxin at levels of either 0.0 or 5.0 μg of aflatoxin per g of diet from 7 to 21 days of age and their response assessed by various physiological parameters.

Body weight, gain, packed red blood cell volume (PCV), plasma albumin, plasma protein and cholesterol responses were significantly reduced from control values by the 5.0 μg/g aflatoxin diet. Males had greater body weights and gains in both dietary regimes than females. Females had significantly higher PCV, protein, albumin and cholesterol values in the 5.0 μg/g aflatoxin group than their male counterparts. These differences resulted in significant sex × aflatoxin level interactions for these parameters. Coefficients of variation were increased for all parameters measured in the 5.0 μg/g aflatoxin treatment compared to values for the control group. This increase was greatest for plasma protein, albumin, and cholesterol responses. Heritabilities were calculated for all responses within both treatment groups and were found to be increased in all cases by the 5.0 μg/g aflatoxin diet. Highly significant phenotypic correlations were determined between body weight and gain and between plasma albumin and total plasma protein in both treatment groups. High phenotypic correlations among PCV, plasma cholesterol, plasma protein, and plasma albumin were noted in the 5.0 μg/g aflatoxin group. Significant genetic correlations were determined between body weight and gain and between plasma albumin and plasma protein in the control group. Body weight and gain and plasma protein, albumin, cholesterol and PCV were genetically correlated in the 5.0 μg/g aflatoxin group. Genetic correlations calculated across environments for the same traits were high for PCV, body weight and gain and much lower for plasma albumin, plasma protein, and plasma cholesterol.

The results of this study demonstrate that genetic variability for resistance to aflatoxin exists in commercial broiler populations. Strong genetic and phenotypic relationships, and high heritabilities associated with plasma albumin and protein suggest their applicability as selection criteria for aflatoxin resistance. Genetic correlation for these traits across dietary environments indicate that responses for aflatoxin resistance should be measured during aflatoxin challenge and suggest that selection for growth and selection for aflatoxin resistance are not antagonistic.

Key words: Heritability – Genetic correlations – Albumin – Protein – PCV – Cholesterol – Body weight – Broiler – Chicken – Aflatoxin

Introduction

Since the outbreak of Turkey "X" disease (Blount 1961) efforts to understand and to ameliorate the detrimental effects of aflatoxicosis have been numerous. Aflatoxin, a secondary metabolite of the mold *Aspergillus flavus*, when administered to poultry results in decreased body weight gains (Smith and Hamilton 1970), anemia (Tung et al. 1975a), severe hypoproteinemia (Tung et al. 1975b) and increased mortality (Smith and Hamilton 1970). The magnitude of these effects have been reported to diminish with age (Lanza et al. 1980a) and to vary among strains and species of poultry (Brown and Abrams 1965; Gumbmann et al. 1970; Lanza et al. 1980b). Moreover, *in vitro* assays using tracheal-organ cultures (Williams et al. 1980) have confirmed the variability of aflatoxin resistance among poultry strains.

Progress in genetic selection for resistance to aflatoxin has been reported in Japanese quail (*Coturnix coturnix japonica*) by breeding from the survivors of an acute aflatoxin challenge (Marks and Wyatt 1979). Quail selected five generations for survival of an orally intubated 3.0 mg/kg body weight aflatoxin challenge were 8.3 times more resistant to aflatoxin induced mortality than were nonselected controls receiving the same aflatoxin challenge.

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The relationship between body weight, packed red blood cell volume (PCV), and plasma protein response with mortality induced by aflatoxin have been assessed (Lanza et al. 1981). Severe declines in plasma protein levels were found to be associated with mortality during aflatoxicosis. These results suggest that plasma protein concentration during aflatoxicosis may be useful in selection for genetic resistance to aflatoxin. This hypothesis is supported indirectly by many studies which have indicated that aflatoxin primarily inhibits protein synthesis at both the transcriptional (Swenson et al. 1975; Swenson et al. 1977; Yu 1977) and translational levels (Pong and Wogan 1969; Sarasin and Moule 1975).

Although the studies with quail clearly indicate that genetic resistance to aflatoxin can be achieved by insulting the organism with massive dosages of aflatoxin and breeding from survivors, this is not a feasible approach to practical selection in poultry populations. The purpose of this study was to assess the genetic variability of response to aflatoxin using physiological parameters known to be affected by aflatoxin and to compare correlations among these responses.

Materials and Methods

A commercial pedigreed broiler population, in which selection had been relaxed after several generations of selection for growth, was utilized. Thirty-one sire families with six to ten dams per sire produced 1464 progeny which were distributed within sex and full-sib family into two treatment groups. All chicks were placed on the University of Georgia broiler starter mash (medicated for coccidiosis control) from 1 to 7 days of age. Dietary aflatoxin at levels of either 0 or 5.0 μg of aflatoxin per g of diet was administered to chicks ad libitum from 7 to 21 days of age. Water was provided ad libitum throughout the study. All families within each level of aflatoxin were reared together in the same floor pen of an environmentally controlled house. The two aflatoxin groups were in the same room but separated by a wire partition.

Aflatoxin used in the study was produced by *Aspergillus parasiticus* NRRL 2999 on sterile polished rice as previously cited (Marks and Wyatt 1979). High pressure liquid chromatographic analysis revealed the relative percentages of aflatoxin metabolites to be 81% aflatoxin B_1 and 19% aflatoxin G_1; other metabolites were present in trace amounts. Appropriate amounts of dried powder were added to the University of Georgia broiler starter ration (medicated for coccidiosis control) to attain the desired dietary levels.

Body weights were measured at 7 and 21 days of age and gains calculated. At 21 days all chicks were bled by cardiac puncture, and the blood was placed in dried, heparinized tubes. Packed cell volumes (PCV) were determined by the microhematocrit method (Johnson 1955). The plasma was separated by centrifugation and frozen for later analysis of total plasma protein (Wooton 1964), plasma albumin (Doumas et al. 1971), and total plasma cholesterol (Zlatkis et al. 1953).

The data were analyzed using either the Statistical Analysis System (SAS 79.3B) (Barr et al. 1976) or the Mixed Model Least-Squares and Maximum Likelihood (LSML76) (Harvey 1977) computer programs. Heritabilities and genetic correlations within each level of aflatoxin were calculated based on half-sib analysis using the sire components of variance of the following model:

$$Y_{ijk} = \mu + a_i + b_j + c_{ij} + e_{ijk}$$

where μ is the common mean; a_i is the effect of the i^{th} sire and is random; b_j is the effect of the j^{th} sex and is fixed; c_{ij} is the random interaction of the i^{th} sire and j^{th} sex; e_{ijk} is individual variation. The percentage of the total variation accounted for by the sire variance component for each parameter in both the control and aflatoxin groups were determined. Genetic correlations across levels of aflatoxin were calculated as described by Yamada (1962) and Benyshek (1979) using a two way classification model:

$$Y_{ijk} = \mu + a_i + b_j + c_{ij} + e_{ijk}$$

where μ is the common mean; a_i is the effect of the i^{th} level of aflatoxin; b_j is the effect of the j^{th} sire; c_{ij} is the interaction of the i^{th} level and j^{th} sire; and e_{ijk} is random error. Sampling variances for these correlations were calculated as described by Robertson (1959). Individual responses were used with data for sexes pooled as described by Massey and Benyshek (1981).

Results and Discussion

Response to Aflatoxin

Aflatoxin significantly reduced average 21 day body weight, 7 to 21 day gain, packed cell volume (PCV), total plasma albumin, cholesterol and protein from control values in a manner similar to previous reports (Smith and Hamilton 1970; Tung et al. 1975a; Tung et al. 1975b; Washburn et al. 1978) (Table 1). Total plasma cholesterol, albumin and protein were reduced from control values by 40, 67 and 55 percent; respectively; whereas, body weight, gain and PCV were reduced by only 9, 11, and 9 percent, respectively. As would be expected, male chicks in both the control and aflatoxin fed groups had significantly higher body weights and greater gains than their female counterparts. Females had significantly higher total plasma cholesterol, protein, albumin and PCV values than males in the 5.0 μg/g aflatoxin group. The differential responses between the sexes noted in the aflatoxin group resulted in significant sex by aflatoxin level interactions for total plasma cholesterol, albumin, protein and PCV. Differential responses to aflatoxin due to sex have also been reported by Williams et al. (1980) using trachealorgan cultures in vitro.

Coefficients of variation for all responses measured were increased by the 5.0 μg/g aflatoxin level; however, no differences due to sex were noted within either aflatoxin level. Coefficients of variation for 21 day body weight, gain, and PCV were increased only 1.2, 1.3 and 1.2 fold, respectively; whereas, total plasma albumin, protein and cholesterol had 4.3, 3.3, and 2.3 fold increases, respectively, (Table 1).

Heritability Estimates

Heritability estimates among the controls were similar to those which have been previously reported. The effects of several generations of previous selection for

Table 1. The effect of dietary aflatoxin and sex on the response of a commercial broiler population

Statistic	Aflatoxin level μg/g	Sex	21 day body weight g	7 to 21 day gain g	PCV %	Plasma albumin mg %	Plasma protein mg %	Plasma cholesterol mg %
Mean	0	M	410x*	326x	25.6x	1337x	2832x	128x
		F	388y	303y	26.0x	1382y	2881x	127x
		M & F	398a**	313a	25.8a	1361a	2859a	127a
	5.0	M	379x	293x	22.9x	411x	1203x	67x
		F	353y	268y	24.2y	486y	1388y	82y
		M & F	364b	279b	23.6b	453b	1306b	75b
C. V.	0	M	13.4	14.9	10.6	10.1	11.9	25.2
		F	12.2	13.5	9.9	10.4	12.2	23.2
		M & F	13.1	14.7	10.3	10.4	12.1	24.1
	5.0	M	15.8	18.5	12.4	45.5	41.2	54.0
		F	15.1	17.7	11.3	42.5	38.0	55.4
		M & F	15.8	18.6	12.1	44.5	40.0	56.0

* xy means within a level of aflatoxin for the same variale with different superscripts are significantly different ($P \leqq 0.05$)
** ab means within a variable for the same statistic with different superscripts are significantly different ($P \leqq 0.05$)

growth characteristics in this population were evidenced by slightly lower heritability estimates for body weight and gain than might be expected. The heritability estimates for PCV, (0.45), was similar to the 0.39 value previously reported for the Athens-Canadian population (Washburn 1967). Total plasma protein and albumin and plasma cholesterol (Cherms et al. 1960) heritability estimates have been previously reported and are in agreement with those reported here.

Heritability estimates for all parameters were increased by the 5.0 μg/g aflatoxin treatment (Table 2). Heritabilities for 21 day body weight and gain were increased from 0.29 and 0.28 in the control group to 0.44 and 0.45, respectively, in the group fed aflatoxin. Only slight differences between heritabilities of the control and aflatoxin groups were noted for PCV and plasma albumin estimates. The heritability for total plasma cholesterol in the 5.0 μg/g aflatoxin group was increased 1.7 fold over the control value but was the lowest heritability estimate among the responses measured. The heritability for total plasma protein was increased by the greatest extent over the control values, 0.21 to 0.43.

The increase in the heritability estimates in the 5.0 μg/g aflatoxin group resulted from an increase in the additive genetic variation for each parameter (Table 2). The ratios of the percent of the total variation accounted for by the sire variance compo-

Table 2. The effect of dietary aflatoxin on half-sib heritabilities and additive genetic variance estimated by sib-analysis for a commercial broiler population

Variable[a]	Level of aflatoxin (μg/g)				
	0.0		5.0		$\Delta\%\ \sigma_s^2$
	$h^2 \pm SE$	$\%\ \sigma_s^2$	$h^2 \pm SE$	$\%\ \sigma_s^2$	
Body weight	0.291±0.13	7.3	0.445±0.15	11.1	1.52
Gain	0.281±0.13	7.0	0.446±0.15	11.1	1.59
PCV	0.452±0.16	11.3	0.473±0.15	11.8	1.04
Albumin	0.478±0.09	11.9	0.525±0.09	13.1	1.10
Cholesterol	0.102±0.16	2.5	0.170±0.16	4.2	1.68
Protein	0.208±0.11	5.2	0.426±0.14	10.6	2.04

[a] Body weight = 21 day body weight; Gain = 7 to 21 day weight gain; PCV = packed cell volume; Cholesterol = total plasma cholesterol; Albumin = total plasma albumin; Protein = total plasma protein;
$h^2 \pm SE$ = heritability ± standard error of the estimate
$\%\ \sigma_s^2$ = percentage of total variation accounted for by the sire variance component $(\sigma_s^2/\sigma_s^2+\sigma_s^2)\times 100$
$\Delta\%\ \sigma_s^2$ = ratio of the percentage of the total variance accounted for by the sire variance component in the aflatoxin group to that of the control group

nents in the aflatoxin group to that of the control indicate the relative increase in additive genetic variation. Body weight, gain, plasma cholesterol and plasma protein exhibited the greatest increases while the increases associated with PCV and plasma albumin were less dramatic.

Phenotypic Correlations

Phenotypic correlations were determined among all traits measured within each level of aflatoxin (Table 3). Highly significant correlations between 21 day body weight and gain were noted in both treatment groups. However, other phenotypic correlations with 21 day body weight, although occassionally statistically significant, were of very low magnitude. The phenotypic correlations of PCV with other responses in the contol group were small; however, in the aflatoxin group, PCV was moderately correlated with plasma cholesterol and to a greater extent with plasma albumin and plasma protein levels. A highly significant phenotypic correlation was observed between plasma albumin and plasma protein in the control group which was approximately two times greater in the aflatoxin group. Plasma albumin was also phenotypically correlated with plasma cholesterol in the aflatoxin group; whereas, this correlation was considerably less in the control group. Similarly, total plasma protein and cholesterol were phenotypically correlated to a much greater degree in the aflatoxin treatment group than in the control group. Body weight gains were not phenotypically correlated with plasma albumin, protein or cholesterol within either level of aflatoxin treatment.

The high phenotypic correlations between 21 day body weight and 7 to 21 day gain were expected. The slight, but significant, correlations between PCV and body weight gain were interesting since this populations has been previously shown to have a lower normal PCV than other commercial or randombred populations (Washburn et al. 1978). Since albumin is a primary constituent of plasma proteins, their high phenotypic correlation in the control group was expected. The dramatic increases in this correlation and the albumin: cholesterol and protein: cholesterol correlation point to the interrelationship of these responses during aflatoxicosis.

Previous studies have clearly demonstrated the interaction of aflatoxin B_1 with guanine nucleosides and RNA polymerases and the resulting inhibition of DNA transcription (Swenson et al. 1975; Swenson et al. 1977; Yu 1977). Moreover, induced dissociation or inhibited reassociation of ribosomes with the endoplasmic reticulum of hepatocytes by aflatoxin B_1 (Pong and Wogan 1969; Sarasin and Moule 1975) has been shown to inhibit the translation of plasma proteins, including albumin and the apoprotein moieties of hepatically derived lipoproteins. Since cholesterol esterified in lipoproteins is transported from hepatocyte to plasma, decreased plasma cholesterol concentrations should be related to decreased plasma protein synthesis induced during aflatoxicosis.

Table 3. The effect of dietary aflatoxin on phenotypic correlations within a commercial broiler population

Variables[b]		Aflatoxin level (μg/g)			
		0.0		5.0	
		$r_{x_1x_2}$	p[a]	$r_{x_1x_2}$	P
BW:	Gain	0.985	0.001	0.989	0.001
	PCV	−0.166	0.001	−0.094	0.014
	Chol.	0.005	0.911	0.019	0.608
	Alb.	0.003	0.935	−0.093	0.016
	Prot.	0.079	0.060	−0.144	0.003
PCV:	Gain	−0.161	0.001	−0.107	0.005
	Chol.	0.062	0.146	0.272	0.001
	Alb.	0.129	0.002	0.500	0.001
	Prot.	0.149	0.004	0.537	0.001
Alb:	Prot.	0.449	0.001	0.886	0.001
	Chol.	0.139	0.001	0.506	0.001
	Gain	0.012	0.781	−0.107	0.006
Prot:	Chol.	0.107	0.012	0.453	0.001
	Gain	0.091	0.032	−0.132	0.001
Chol:	Gain	0.009	0.832	0.002	0.947

[a] P = probability of significance that the correlation is significantly different from zero

[b] BW = 21 day body weight; Gain = 7 to 21 day gain; PCV = packed cell volume; Chol. = total plasma cholesterol; Alb. = total plasma albumin; Prot. = total plasma protein

Genetic Correlations

The magnitude of genetic correlations were similar to those observed among the phenotypic correlations (Table 4). As might be expected, 21 day body weights and gains were highly correlated genetically in both the aflatoxin and control groups; however, 21 day body weight was not highly correlated with other responses within either level of aflatoxin. Gain was not genetically correlated with plasma albumin or cholesterol in either treatment group. Some genetic relationship was noted between total plasma protein and gain in the control group, but not among the aflatoxin treated chicks. PCV was not genetically correlated with other responses among the control chicks but was slightly correlated with plasma albumin and significantly correlated with plasma protein among the aflatoxin treated chicks. As noted among the phenotypic correlations, a high genetic correlation was determined between plasma albumin and protein in the control group. This correlation was greatly enhanced in the aflatoxin treated group. Moreover, high genetic correlations among total plasma cholesterol, albumin and

Table 4. The effect of dietary aflatoxin on genotypic correlations within a commercial broiler population

Variables[a]		Aflatoxin level (μg/g)			
		0.0		5.0	
		$r_{g_1g_2}$	SE	$r_{g_1g_2}$	SE
BW21:	Gain	0.989	0.008[b]	0.997	0.002[b]
	PCV	−0.159	0.300	−0.219	0.250
	Chol	0.431	0.442	0.251	0.312
	Alb	−0.383	0.271	0.198	0.248
	Prot	0.408	0.324	0.222	0.256
PCV:	Gain	−0.147	0.302	−0.201	0.252
	Chol	−0.175	0.406	−0.087	0.317
	Alb	0.163	0.263	0.267	0.230
	Prot	0.175	0.317	0.422	0.211[b]
Alb:	Prot	0.692	0.202[b]	0.942	0.034[b]
	Chol	−0.159	0.398	0.938	0.124[b]
	Gain	−0.289	0.283	0.247	0.245
Prot:	Chol	0.117	0.488	0.918	0.144[b]
	Gain	0.533	0.310	0.259	0.254
Chol:	Gain	0.446	0.445	0.279	0.310

[a] BW 21 = 21 day body weight; Gain = 7 to 21 day gain; PCV = packed cell volume; Chol = total plasma cholesterol; Alb. = total plasma albumin; Prot = total plasma protein

[b] Probability of significance that the genetic correlation is significantly different than zero ($P < 0.05$)

protein were noted with the 5.0 μg/g aflatoxin group but not in the control group.

The enhancement of the genetic correlations among plasma protein, cholesterol, and albumin in the 5.0 μg/g aflatoxin group illustrates the genetic relationship between these responses during aflatoxicosis. Since breeding from survivors of a massive aflatoxin challenge has been found to be an effective method of selection for aflatoxin resistance (Marks and Wyatt 1979) and since severely declining plasma protein response during aflatoxicosis has been reported to be predictive of impending mortality (Lanza et al. 1981), the high genetic correlations among plasma protein and albumin and the high heritabilities of these responses during aflatoxicosis suggest that selection for resistance to aflatoxin by selecting for high plasma protein or a correlated response, such as plasma albumin, is feasible. Moreover, since growth responses were not negatively correlated with aflatoxin resistance, selection for growth in a normal environment from chicks being challenged with aflatoxin to assess their resistance should be possible. This point is more clearly illustrated by genetic correlations calculated for the same trait across both aflatoxin environments.

By regarding a character measured in two environments as different characters with genetic correlation between them, the extent to which the physiological mechanisms differ and therefore, the extent to which the genes expressed differ, may be assessed by the magnitude of the genetic correlation between the characters (Falconer and Latyszewski 1952; Falconer 1960). High genetic correlations between environments were determined for body weight, gain and PCV; whereas, lower genetic correlations were determined for plasma cholesterol, protein, and albumin (Table 5). These results clearly indicate a low sire × level of aflatoxin interaction for growth response and suggest that some normally high growth families were aflatoxin susceptible, experiencing greater than average growth depression during aflatoxicosis, and conversely, some low growth families were more resistant to the toxin, experiencing less than average growth depressions. A very high genetic correlation for PCV was also noted, lending further credence to the contention that aflatoxin associated anemia is a manifestation of the severe hypoproteinemia induced by aflatoxin rather than a direct cause and effect relationship. Plasma cholesterol, albumin and protein had low genetic correlations between the two environments indicating that the expression of the genes normally controlling these characters is directly affected by the interaction of other gene products affecting aflatoxin metabolism.

The degree to which hepatic protein synthesis is inhibited by aflatoxin is a function of both the specific activity of mixed function oxidases of the microsomal enzyme system to convert the aflatoxin metabolite to an epoxide capable of binding guanine (Swenson et al. 1977; Yu et al. 1977) and/or the ability of hepatocytes to conjugate aflatoxin to bile salts via the glutathione system and remove it from the liver (Raj et al. 1975). These potential areas of genetic dissimilarity within the

Table 5. The genetic correlation across aflatoxin levels for the same parameter in each environment

Variable[a]	Genetic correlation	
	$R_{g_1g_2}$	Var $(R_{g_1g_2})$[c]
Body weight	0.78	0.06
Gain	0.75	0.06
PCV	0.94	0.01
Albumin	0.44	0.07
Protein	0.33	0.15
Cholesterol	0.32	0.39

[a] Body weight = 21 day body weight; Gain = 7 to 21 day gain; PCV = packed cell volume; Albumin = total plasma albumin; Protein = total plasma protein; Cholesterol = total plasma cholesterol

[b] $R_{g_1g_1}$ = Genetic correlation across environments

[c] Var $(R_{g_1g_2})$ = Sampling variance of genetic correlation (N = 1,225)

populations and possibly others regulate the damaging effects of the toxin within hepatocytes and therefore the relative resistance of the individuals. This study clearly indicates that selection for aflatoxin resistance must be conducted in an aflatoxin stress environment and that total plasma protein or another highly correlated trait may be used as a selection criterion. The rapid progress reported by Marks and Wyatt (1979) in selecting for aflatoxin resistance in quail and the high heritabilities of plasma protein and albumin response in the aflatoxin stress environment, suggest that progress using these selection criteria would also be rapid.

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