The influence of vitamin A status on the response of chickens to aflatoxin B_1 and changes in liver lipid metabolism associated with aflatoxicosis

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1 A series of experiments were conducted to investigate the effects of dietary retinol status on chickens ingesting aflatoxin B_1. The effects of dietary supplementation with biotin and α-tocopherol were also examined.

2. Aflatoxin B_1 levels greater than 1 mg/kg diet had a detrimental effect on 'liveability', body-weight gain, food intake and food conversion efficiency. When fed for more than 2 weeks aflatoxin increased relative liver weight and liver lipid concentration. These effects were less pronounced with avitaminotic A chickens.

3. A synergistic effect on hydropericardium development was observed between aflatoxin B_1 and retinol. This effect was not observed when the dietary level of α-tocopherol was increased tenfold.

4. The specific activities of certain hepatic lipogenic and amino acid-metabolizing enzymes were influenced by aflatoxin ingestion. A reduction in lipogenic enzyme activity was observed before a reduction in the activities of amino acid-metabolizing enzymes.

5. Liver fatty acid composition was significantly influenced by aflatoxin B_1. The extent of these changes was reduced by the inclusion of additional dietary biotin.

Toxigenic strains of the fungus *Aspergillus flavus*, which are widespread in animal feedstuffs (Bryden *et al.* 1975) can produce secondary metabolites called aflatoxins. Ingestion of these toxins, particularly aflatoxin B_1, decreases growth rate and food conversion efficiency and increases mortality (Wogan, 1966; Allcroft, 1969; Smith & Hamilton, 1970). Fatty infiltration of the liver has also been observed in animals and poultry fed on aflatoxin B_1 (Newberne & Butler, 1969; Smith & Hamilton, 1970).

Numerous studies have shown that the detrimental effects of aflatoxin in animals and birds can be markedly influenced by diet (see Hamilton, 1977). However, the effect of dietary vitamin status is still not clear. Fortification of a broiler diet with a fourfold excess of vitamins did not reduce the growth inhibitory effect of aflatoxin (Hamilton *et al.* 1974) whereas Knake *et al.* (1973) observed greater weight gains in quail (*Coturnix coturnix*) after feeding additional vitamins in a diet containing aflatoxin.

The interaction of aflatoxin with single-vitamin dietary modifications is more definite. Hamilton *et al.* (1974) have shown that the response of chickens to a single vitamin deficiency is dependent on the vitamin in question. Diets deficient in riboflavin and cholecalciferol made chickens more sensitive to aflatoxin. However, deficiencies of menaphthone and α-tocopherol had no influence, while a thiamin deficiency had a protective effect against the growth inhibitory effect of aflatoxin (Hamilton *et al.* 1974).

Lowered liver levels of vitamin A in calves (Allcroft & Lewis, 1963), pigs (Harding *et al.* 1963; Gumbmann & Williams, 1969) and chickens (Carnaghan *et al.* 1966; Kriz, 1970) suggest that aflatoxin modifies vitamin A metabolism. The experiments described in this study were planned to investigate the interaction of dietary retinol status and aflatoxin. In

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addition, chickens ingesting aflatoxin and different dietary levels of retinol were also fed on diets fortified with biotin and α-tocopherol. Biotin was investigated as it has been shown to be involved in the aetiology of certain fatty liver conditions (Marchetti & Puddu, 1964; Marchetti *et al.* 1966; Payne *et al.* 1974; Whitehead *et al.* 1974; Balnave *et al.* 1977), but there have been no reports of the influence of biotin upon aflatoxicosis. Although no interaction of α-tocopherol and aflatoxin has been demonstrated (Todd *et al.* 1968; Hamilton *et al.* 1974) it was included in this study as excess α-tocopherol has been shown to alleviate the harmful effects of hypervitaminosis A (McCuaig & Motzok, 1970).

As no detailed examination of the enzymic changes associated with aflatoxin-induced fatty liver development has been made the opportunity was taken to examine the changes in some hepatic enzyme activities in birds fed on aflatoxin B_1.

MATERIALS AND METHODS

Birds

Debeaked, commercial broiler chickens, 1-d-old, were obtained from a local hatchery. Male chickens were used in all experiments except Expt 5. The chickens were placed in cages under continuous illumination. Heating was provided to 3 weeks of age. In Expts 1, 3 and 5 they were fed on a commercial chick diet from hatch until placed on experimental diets. In Expts 2 and 4 experimental diets were fed from 1-d-old.

Aflatoxin production and diets

An aflatoxigenic strain of *Aspergillus flavus* isolated locally from barley and deposited in the Commonwealth Mycological Institute, where it was designated IMI 157979, was cultured on polished long-grain rice in 4 l plastic containers. After addition of 1 kg rice and 500 ml water the containers were autoclaved for 15 min at a pressure of $1{\cdot}0 \times 10^5$ N/m^2 before inoculation with *A. flavus* colonies. The inoculated containers were incubated at 25° for 16 d and shaken manually every other day. After the incubation period the containers were again autoclaved and the contents removed and dried at 60° for 15 h. Aflatoxin levels were determined by the method of Pons *et al.* (1972) as described by Bryden *et al.* (1975).

The composition of the basal experimental diet is shown in Table 1. Aflatoxicosis was induced in chickens by replacing part of the wheat component with aflatoxin-contaminated rice to give the desired dietary level of aflatoxin. A corresponding amount of non-toxic rice replaced wheat in corresponding aflatoxin-free diets. Retinol-supplemented diets were produced by adding suitable amounts of stabilized retinyl ester beadlets (Rovimix A-500; Roche Products Pty Ltd) equivalent to 1·8 and 18 mg retinol/kg diet.

Experimental

In all experiments food and water were provided *ad lib.* Chickens were weighed and food consumption recorded at weekly intervals. Chickens that died during the experiment were post-mortemed and aflatoxicosis diagnosed by macroscopic lesions as described by Newberne (1973).

In Expt 1 duplicate groups of twenty 7-d-old chickens were fed on diets containing 0, 0·3, 1·25 or 2·0 mg aflatoxin B_1/kg and supplemented with either 1·8 or 18 mg retinol/kg. After 3 weeks three chickens, randomly selected from each group, were killed by cervical dislocation and the livers rapidly removed and weighed. The experimental diets were removed and the remaining birds placed on a commercial diet. The experiment was terminated when the chickens were 7 weeks of age.

In Expt 2, groups of fifteen 1-d-old chickens were placed on diets supplemented with either 0, 1·8 or 18 mg retinol/kg. Two groups of chickens fed on each level of retinol also

Table 1. *Composition (g) of basal diet*

Ingredients	
Wheat	720
Soya-bean meal	150
Meat meal	130
Vitamin-mineral premix (g/kg)*	25

* The premix supplied (/kg diet); cholecalciferol 62·5 μg, α-tocopheryl acetate 30 mg, menaphthone 2·1 mg, cyanocobalamin 6 μg, nicotinic acid 10 mg, pantothenic acid 7·5 mg, pteroylmonoglutamic acid 1 mg, riboflavin 4 mg, pyridoxine hydrochloride 0·82 mg, choline chloride 350 mg, cobalt chloride 0·15 mg, copper sulphate 2 mg, iodine 0·75 mg, ferric chloride 20 mg, manganese sulphate 150 mg, molybdenum sulphate 0·4 mg, zinc sulphate 50 mg, ethoxyquin 75 mg, 3-nitro-4-hydroxyphenylarsonic acid 45 mg.

received 0, 0·5 or 2·0 mg aflatoxin B_1/kg diet from 1-d-old while two equivalent groups received these aflatoxin-contaminated diets from 21 d of age. In each instance the aflatoxin-contaminated diets were fed for 3 weeks. After this period six chickens were killed from each treatment and the livers removed.

In Expt 3, groups of twelve chickens were fed on diets supplemented with either 1·8 or 18 mg retinol/kg. Six groups of chickens fed on each level of retinol also received either 0 or 200 μg biotin/kg diet (Rovimix H; Roche Products Pty Ltd). Three of the groups on each of these dietary combinations also received 0 or 1 mg aflatoxin B_1/kg diet. Feeding of these eight experimental diets commenced at 7 d of age. After 28 d two birds from each treatment were killed, the livers weighed and liver lipid content and fatty acid composition determined.

In Expt 4, groups of fifteen chickens were fed on diets supplemented with 0, 1·8 or 18 mg retinol/kg from 1-d-old. At 21 d four groups of chickens fed on each level of retinol received 0 or 2 mg aflatoxin B_1/kg diet; two groups of chickens from each of these dietary combinations also received a dietary supplement of α-tocopherol (Rovimix E-25; Roche Products Pty Ltd) equivalent to 0 or 270 mg α-tocopherol acetate/kg diet to bring total supplements to 30 and 300 mg/kg respectively. At 42 d of age four birds were randomly selected from each treatment and killed by decapitation, the livers were weighed and portions taken for lipid determination. Samples of the livers from birds which did not receive the additional dietary α-tocopherol supplement were also taken for determination of liver biotin and liver nucleic acid concentrations. Portions were also homogenized with 4 vol. ice-cold 0·1 M-Tris buffer, pH 7·4. The homogenates were centrifuged at 18000 *g* for 30 min at 2–5° and the resulting supernatant fractions assayed for enzyme activities as described by Pearce & Balnave (1976). The following enzymes were assayed: ATP citrate lyase (*EC* 4.1.3.8), NADP-malate dehydrogenase (*EC* 1.1.1.40), aspartate aminotransferase (*EC* 2.6.1.1) and alanine aminotransferase (*EC* 2.6.1.2).

In Expt 5 replicated groups of female chickens were fed on the basal diet supplemented with 1·8 mg retinol/kg and either 0 or 2 mg aflatoxin B_1/kg from 21 d of age. Birds were randomly selected from both treatments after 2, 6 and 15 d and killed by decapitation. The livers were rapidly removed, chilled and liver enzyme activities determined as in Expt 4. In addition, glutamate dehydrogenase (*EC* 1.4.1.2) was assayed as described by Pearce & Balnave (1976).

Chemical analyses

Liver and dietary biotin contents were determined by the method of Hood (1975). Liver lipid was extracted (Folch *et al.* 1957) and determined gravimetrically. Liver fatty acid composition was determined by gas-liquid chromatography (Pearson *et al.* 1972). Protein content of cell-free extracts was determined by the Biuret reaction (Layne, 1957). Nucleic acids were extracted from liver tissue using a modification of the Schmidt-Thannhauser

Table 2. *Expt* 1. *Effect of dietary retinol supplementation on body-weight, food conversion efficiency (g food/g body-weight gain), liver weight and mortality of broiler chickens fed different levels of aflatoxin between* 1 *and* 4 *weeks of age*

	Retinol equivalent (mg/kg)	Dietary aflatoxin (mg/kg)				SEM (8 df)
		0·0	0·3	1·25	2·0	
Final body-wt at 28 d (g)	1·8	447	433	360	294	9·87
	18·0	448	437	329	305	
Food intake (7–28 d) (g/bird)	1·8	775	743	640	541	14·52
	18·0	814	752	598	545	
Food conversion (7–28 d)	1·8	2·14	2·14	2·34	2·59	0·12
	18·0	2·23	2·14	2·46	2·45	
No. of deaths (7–28 d)	1·8	0	0	0	0	
	18·0	0	0	2	6	
Liver wt at 28 d (g/kg body-wt)	1·8	35·6	35·4	41·4	42·7	1·0
	18·0	33·4	36·9	47·6	44·7	
Body-wt at 7 weeks of age*	1·8	881	908	750	676	34·00
	18·0	939	884	731	654	

* Body-weight of birds, 3 weeks after removing aflatoxin-contaminated food.

method as described by Munro & Fleck (1966). RNA and DNA were determined by the methods of Giles & Myers (1965) and Hatcher & Goldstein (1969) respectively.

Statistical analyses

The results of the studies were analysed by analysis of variance and the Student's *t* test. 'Mortality' results are given in the tables but statistical analyses were not carried out as in many instances no variation was found between replicates of the same treatment (Snedecor & Cochran, 1967).

RESULTS

Throughout the text references to liver weight refer to values calculated relative to body weight (i.e. g liver/kg body-weight). Similarly, levels of liver constituents are given on a liver weight basis.

During these studies post-mortem examination of chickens consuming aflatoxin B_1-contaminated diets indicated the presence of pale, swollen livers and kidneys. Mortality was increased in avitaminotic A chickens and in chickens ingesting aflatoxin B_1. In the latter instance mortality tended to increase when birds also received the highest level of supplementary retinol and approximately 20 % of these chickens had a hydropericardium.

The results of Expt 1 are shown in Table 2. Aflatoxin significantly reduced body-weight ($P < 0{\cdot}001$), food intake ($P < 0{\cdot}001$) and food conversion efficiency ($P < 0{\cdot}05$) but increased liver weight ($P < 0{\cdot}001$). These effects were most noticeable at the two highest dietary aflatoxin concentrations. At these two levels of aflatoxin the higher level of dietary retinol significantly ($P < 0{\cdot}05$) increased liver weight and reflected an over-all ($P < 0{\cdot}05$) aflatoxin × retinol interaction. Vitamin A supplementation to 18 mg retinol/kg had no over-all effect on body-weight, food intake or food conversion efficiency. Aflatoxin ($P < 0{\cdot}001$) but not retinol, significantly influenced body-weight gain during the 3 weeks after the removal of the aflatoxin-contaminated diets.

Table 3. *Expt 2. Response of chickens fed on diets containing different levels of retinol from 1 d of age and different levels of aflatoxin from 1 to 21 d of age or 21 to 42 d of age*

Period on aflatoxin (d of age)	Retinol equivalent (mg/kg)	Aflatoxin content (mg/kg)	Final body-wt (g)	Food intake (g/bird)	Food conversion (g food/g body-wt gain)	Liver wt (g/kg body-wt)	No. of deaths
1–21	0	0·0	295	484	1·88	32	3
		0·5	295	479	1·87	37	0
		2·0	227	402	2·12	41	4
	1·8	0·0	337	520	1·74	35	0
		0·5	320	507	1·80	40	0
		2·0	235	424	2·15	49	5
	18·0	0·0	336	532	1·80	36	0
		0·5	331	542	1·86	39	0
		2·0	222	396	2·14	55	5
21–42	0	0·0	504	906	3·36	33	10
		0·5	637	1187	2·97	27	5
		2·0	608	1050	2·93	30	5
	1·8	0·0	1052	1658	2·21	24	0
		0·5	1052	1654	2·19	25	0
		2·0	901	1495	2·50	38	1
	18·0	0·0	1047	1682	2·28	26	0
		0·5	1034	1649	2·26	27	1
		2·0	879	1474	2·60	46	3
SEM (18 df)			13·4	21·5	0·07	2·8	—

The results of Expt 2 (Table 3) indicate that the high level of aflatoxin reduced body-weight, food intake and food conversion ($P < 0{\cdot}001$) while increasing liver weight ($P < 0{\cdot}001$). Retinol status significantly affected body-weight, food intake, food conversion ($P < 0{\cdot}001$) and liver weight ($P < 0{\cdot}05$). This was due primarily to the different response of the chickens receiving no retinol. There were significant interactions (period of experiment × aflatoxin, $P < 0{\cdot}05$; period of experiment × vitamin A, $P < 0{\cdot}001$) with regard to body-weight, food intake and food conversion. The higher level of significance observed with retinol presumably reflects the gradual onset of a vitamin A deficiency during the first 3 weeks of the experiment. At 42 d of age chickens fed on the retinol-deficient diet exhibited typical vitamin A deficiency symptoms including high mortality, general weakness, emaciation, staggering gait, ruffled plumage and keratinization of the third eyelid (Scott *et al.* 1969).

In Expt 3 (Table 4) aflatoxin reduced ($P < 0{\cdot}001$) body-weight, food intake and food conversion efficiency. Responses were not significantly affected by retinol and biotin supplementation and no significant interactions were observed. Chickens fed on aflatoxin had enlarged livers ($P < 0{\cdot}001$) with increased lipid content ($0{\cdot}1 > P > 0{\cdot}05$). Liver fatty acid composition was significantly influenced by aflatoxin with increased levels of 14:0 ($P < 0{\cdot}05$), 16:0 ($P < 0{\cdot}05$) and 18:1 ($P < 0{\cdot}01$) fatty acids and decreased levels of 20:4 ($P < 0{\cdot}001$). Dietary biotin supplementation reduced the extent of these changes when given without 18 mg retinol/kg diet.

The results of Expt 4 are given in Table 5. Aflatoxin reduced body-weight, food intake ($P < 0{\cdot}001$) and food conversion efficiency ($P < 0{\cdot}01$) but increased liver weight and liver lipid content ($P < 0{\cdot}001$). Retinol supplementation influenced body-weight ($P < 0{\cdot}001$), food intake ($P < 0{\cdot}001$), food conversion efficiency ($P < 0{\cdot}01$) and liver lipid content ($P < 0{\cdot}01$). Again there was a difference in the nature of the responses between chickens fed

Table 4. *Expt* 3. *Response of chickens to diets* containing aflatoxin* (1 *mg* B_1/*kg*) *and supplements of retinol* (18 *mg/kg*) *and biotin* (200 *μg/kg*) *given from* 1 *to* 5 *weeks of age*

Dietary treatment ...	None	Retinol	Biotin	Retinol + biotin	Aflatoxin	Aflatoxin + retinol	Aflatoxin + biotin	Aflatoxin + retinol + biotin	SEM (16 df)	SEM (8 df)
Final body-wt (g)	633	627	631	613	375	368	394	373	14·0	—
Food intake (g/bird)	1206	1222	1248	1173	864	917	952	1008	39·1	
Food conversion (g food/g body-wt gain)	2·15	2·20	2·22	2·16	2·84	3·06	2·92	3·28	0·13	—
No. of deaths	0	1	2	0	2	2	3	6	—	—
Liver wt (g/kg body-wt)	26·9	25·1	23·8	25·4	42·6	40·0	38·7	45·9	—	3·9
Liver lipid (mg/g liver)	42·2	45·6	46·1	48·0	78·3	79·9	48·2	82·7	—	17·6
Liver fatty acid composition (mg/g total fatty acids)										
14:0	5	5	6	5	8	8	6	10	—	1·2
16:0	198	168	186	179	252	220	196	240	—	22·8
16:1	22	14	15	25	35	31	16	42	—	9·8
18:0	181	213	226	189	165	170	216	172	—	25·2
18:1	195	167	189	189	396	345	263	328	—	45·2
18:2	119	114	122	106	84	90	128	93	—	19·1
20:4	118	130	123	110	84	38	74	55	—	16·0
22:6	46	40	71	53	35	39	35	35	—	15·9

* Basal diet (see Table 1) supplemented with 1·8 mg retinol/kg.

Table 5. *Expt* 4. *Response of chickens fed on diets containing different levels of retinol from* 1 *d of age and different levels of* α*-tocopherol and aflatoxin from* 21 *to* 42 *d of age*

α-Tocopheryl equivalent (mg/kg)	Retinol equivalent (mg/kg)	Dietary aflatoxin (mg/kg)	Final body-wt (g)	Food intake (g)	Food conversion (g food/g body-wt gain)	Liver wt (g/kg body-wt)	Liver lipid (mg/g liver)	No. of deaths
30	0	0	722	1075	2·45	31·8	41·8	9
		2	723	1139	2·64	39·8	90·9	7
	1·8	0	1028	1811	2·51	33·0	46·6	1
		2	864	1438	2·63	40·8	141·3	3
	18·0	0	969	1632	2·47	29·8	37·0	2
		2	881	1536	2·71	44·5	184·3	6
300	0	0	834	1290	2·27	27·3	28·3	7
		2	814	1310	2·30	31·8	47·2	5
	1·8	0	1035	1721	2·49	24·3	27·0	1
		2	828	1350	2·85	39·8	114·5	1
	18·0	0	949	1661	2·49	25·0	32·6	1
		2	823	1311	2·79	45·8	144·1	3
SEM (12 df)		—	30·3	56·1	0·08	—	—	—
SEM (36 df)		—	—	—	—	2·9	19·6	—

on the basal diet (Table 1) compared with both retinol-supplemented diets. This was reflected in a significant aflatoxin × retinol interaction with regard to body-weight ($P < 0·01$), food intake ($P < 0·001$), liver weight ($P < 0·05$) and liver lipid content ($P < 0·01$). α-Tocopherol supplementation as 300 mg α-tocopheryl acetate/kg reduced liver weight and liver lipid content ($P < 0·05$) and there was a significant retinol × α-tocopherol interaction with regard to body-weight ($P < 0·05$), food intake ($P < 0·01$) and food conversion efficiency ($P < 0·05$).

The liver enzyme analyses from birds not receiving the additional α-tocopherol supplement in Expt 4 are given in Table 6. These indicate that aflatoxin reduced the specific activity of hepatic ATP citrate lyase ($P < 0·05$) and NADP-malate dehydrogenase ($P < 0·001$).¶ Liver DNA ($P < 0·001$) and liver RNA ($P < 0·001$) concentrations and the liver RNA:DNA ($P < 0·001$) were reduced by aflatoxin. Dietary vitamin A status significantly influenced liver DNA ($P < 0·01$) and RNA ($P < 0·001$) concentrations.

The biotin level of the basal diet in Expt 4 was 145 μg/kg. Aflatoxin, but not retinol, reduced ($P < 0·05$) liver biotin concentrations when determined on a liver weight basis. This effect was not observed when values were expressed relative to DNA, except in the avitaminotic A chickens.

The results of Expt 5 are shown in Table 7. These results indicate that feeding aflatoxin B_1 for 2 or 6 d had no significant effect on liver weight or liver lipid content. However, substantial reductions were observed at both 2 and 6 d in the specific activities of the hepatic lipogenic enzymes ATP citrate lyase and NADP-malate dehydrogenase. These reductions attained significance ($P < 0·05$) with ATP citrate lyase on day 2 and NADP-malate dehydrogenase on day 6. No significant effect was observed on the specific activities of the hepatic transaminases or glutamate dehydrogenase at either day 2 or day 6.

Feeding aflatoxin B_1 for 15 d substantially increased liver weight and liver lipid content although these increases did not attain significance because of the large variation between birds fed on aflatoxin. The reductions in hepatic lipogenic enzyme activity observed after 2 and 6 d of feeding aflatoxin were not evident after 15 d. The specific activities of hepatic

Table 6. *Expt 4. Specific activities (nmol substrate metabolized/min per mg protein in the extract) of some hepatic enzymes and hepatic nucleic acid and biotin content in chickens given different amounts of retinol from 1 d and different amounts of aflatoxin from 21 to 42 d of age*

Retinol equivalent (mg/kg)	Dietary aflatoxin (mg/kg)	ATP citrate lyase (*EC* 4.1.3.8)	NADP-malate dehydrogenase (*EC* 1.1.1.40)	Aspartate aminotransferase (*EC* 2.6.1.1)	Alanine aminotransferase (*EC* 1.6.1.2)
0	0	12·7	124·0	484·5	10·2
	2	10·1	59·8	447·6	7·7
1·8	0	13·7	130·0	412·5	8·7
	2	10·1	75·7	426·9	7·8
18·0	0	14·0	162·4	434·4	12·1
	2	7·8	46·4	356·2	7·2
SEM (18 df)		2·3	8·2	44·0	2·2

		Liver				
					Biotin	
		DNA (mg/g liver)	RNA (mg/g liver)	RNA:DNA	(μg/g liver)	(mg/g DNA)
0	0	1·68	5·13	3·05	3·55	2·11
	2	1·51	2·53	1·68	1·18	0·78
1·8	0	1·62	5·35	3·28	1·91	1·18
	2	1·11	2·07	1·86	1·72	1·55
18·0	0	1·45	4·13	2·85	2·07	1·43
	2	0·65	1·20	1·85	1·39	2·14
SEM (18 df)		0·15	0·21	0·24	0·52	0·41

Table 7. *Expt 5. Liver weight, liver lipid and hepatic enzyme specific activities (nmol substrate metabolized/min per mg protein in the extract) of chicks fed on 0 and 2 mg aflatoxin B_1/ kg diet*

(Mean values with their standard errors; no. of chicks in parentheses)

Period on diets (d)	Dietary aflatoxin (mg/kg)	Liver wt (g/kg body-wt)		Liver lipid (mg/g liver)		ATP citrate lyase (*EC* 4.1.3.8)		NADP-malate dehydrogenase (*EC* 1.1.1.40)		Glutamate dehydrogenase (*EC* 1.4.1.2)		Aspartate aminotransferase (*EC* 2.6.1.1)		Alanine aminotransferase (*EC* 2.6.1.2)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2 (4)	0	41·1	2·2	ND		14·4	0·8	133	2	ND		395	21	18·1	2·6
	2	39·9	1·8	ND		9·6	1·6	140	27	ND		362	15	14·6	1·7
6 (5)	0	29·7	1·0	35·3	1·3	13·3	1·1	207	30	41·5	8·4	482	31	11·8	1·2
	2	31·5	1·9	32·7	2·8	9·1	2·5	124	22	34·6	9·1	515	17	9·9	0·5
15 (5)	0	27·6	1·8	32·0	2·3	16·5	2·4	168	17	83·1	15·6	513	45	29·2	5·3
	2	33·6	3·4	65·2	21·4	18·3	2·8	128	15	48·8	11·5	499	42	15·4	1·1

ND, not determined.

glutamate dehydrogenase and alanine aminotransferase in birds fed on aflatoxin for 15 d were reduced to approximately half those in control birds, this reduction attaining significance ($P < 0.05$) with the latter enzyme.

DISCUSSION

Feeding aflatoxin had a detrimental effect on mortality, body-weight gain, food intake and food conversion efficiency. These effects and the increased liver weight and liver lipid content observed when aflatoxin was fed have been reported previously (Smith & Hamilton, 1970; Hamilton *et al.* 1972). The lack of a growth-depressing effect of aflatoxin in avitaminotic A chickens in Expts 2 and 4 may indicate that these chickens respond differently to aflatoxin than chickens receiving retinol supplements. Such a situation has been observed in rats (Newberne & Rogers, 1973; Reddy *et al.* 1973). The difference in response of vitamin A-deficient members of both species to their adequately-fed controls also ingesting aflatoxin suggests that vitamin A-deficiency may alter aflatoxin metabolism. Becking (1973) and Ferrando *et al.* (1974) found that vitamin A deficiency impairs the activity of liver mixed-function oxidase. Such an impairment may explain the apparently-changed toxicity of aflatoxin as it is now generally accepted (for reviews, see Wogan, 1975; Campbell & Hayes, 1976) that an active metabolite of aflatoxin B_1 is responsible for the alterations in metabolism. Therefore, vitamin A may be added to the class of vitamin deficiencies that have a protective effect during aflatoxicosis.

The failure to observe any differences in response in chickens fed on non-toxic diets and receiving 1·8 or 18·0 mg retinol/kg agrees with the results of Squibb (1963) and Pudelkiewicz, Webster, Olson *et al.* (1964). However, increasing levels of retinol accentuated the increased liver weight and liver lipid content observed when aflatoxin was fed and this contrasts with the beneficial effect of vitamin A deficiency noted previously. Therefore, it is interesting to note that increasing retinol intake to levels much higher than used in this study has been shown to induce fatty infiltration of livers in chickens (Squibb, 1963; Pudelkiewicz, Webster, Olson *et al.* 1964) and rats (Singh *et al.* 1969; Mallia *et al.* 1975).

Aside from the increased liver weight and liver lipid content the only gross lesion observed in birds given 2 mg aflatoxin B_1/kg and 18 mg retinol/kg was a hydropericardium but this lesion was not evident when aflatoxin and retinol were fed separately. Smith & Hamilton (1970) have previously observed a serous infiltration of the pericardium in a field outbreak of broiler aflatoxicosis. They did not report this lesion in laboratory trials with the toxin. McCuaig *et al.* (1972) reported development of hydropericardium in chickens fed equivalent to $10{\cdot}8 \times 10^3$ mg retinol/kg in a 200 g tallow/kg diet and suggested that the lesion was due to a synergistic effect between a toxic factor in the tallow and vitamin A. They also suggested that their condition may have been aggravated by impaired vitamin E absorption which has been shown to occur in the presence of excessive dietary vitamin A (Pudelkiewicz, Webster & Matterson, 1964; Combs, 1976). A relationship between vitamin E deficiency and chick hydropericardium development has also been demonstrated by Bird & Culton (1940). In the present work no instances of hydropericardium were found when high levels of dietary α-tocopherol were used (Expt 4).

The results of the time-course study (Expt 5) indicate that the initial effect of aflatoxin B_1 on liver metabolism was to reduce the specific activities of the lipogenic enzymes ATP citrate lyase and NADP-malate dehydrogenase. This occurred before the depression in protein metabolism as measured by the activities of hepatic alanine aminotransferase and glutamate dehydrogenase, which was observed 15 d after the initial feeding of aflatoxin B_1 in Expt 5 and after 21 d in Expt 4. This response has been reported in other studies (Shank & Wogan, 1966; Clifford & Rees, 1967). It would therefore appear unlikely that the initial

reduction in lipogenic enzyme activity was the result of a depressed formation of enzymes as has been suggested by Donaldson *et al.* (1972). The observation that liver lipid concentration was similar in both groups of chicks 6 d after the introduction of aflatoxin is in agreement with the report of Carnaghan *et al.* (1966) and indicates that a lipid feedback control mechanism resulting from high levels of liver lipid would not explain the initial reduction in lipogenic enzyme activity in birds fed on aflatoxin. The similarity in lipogenic enzyme activity in both groups of birds after 15 d in Expt 5 also mitigates against a depressed formation of lipogenic enzymes in birds fed aflatoxin.

The present studies indicate that extended feeding with aflatoxin increased liver weight and liver lipid content. However, the enzyme studies in Expts 4 and 5 indicate that these effects were not associated with increased hepatic lipogenesis. In Expt 4 the reduction in lipogenic enzyme activity was most noticeable at the high level of retinol supplementation where the increases in liver weight and liver lipid were greatest. The specific activities of the hepatic amino acid-metabolizing enzymes aspartate aminotransferase and alanine aminotransferase were also substantially reduced by aflatoxin at the high level of retinol supplementation. Therefore, the increase in liver lipid content in birds fed on aflatoxin B_1 for an extended period are likely to be the result of reduced lipid transport from the liver. Such a reduction in lipid transport has been demonstrated in chickens by Tung *et al.* (1972). Decreased lipid content in extra-hepatic tissues in chickens fed on aflatoxin (Wyatt Thaxton & Hamilton, 1975; W. L. Bryden, unpublished results) reinforces this suggestion.

A reduction in lipogenic enzyme activity may not necessarily mean that the potential for lipogenesis is decreased but only that high levels of liver lipid, resulting from a reduction in lipid transport, are exerting a negative feedback control on lipogenic enzyme activity. Such a condition, with similar changes in liver lipid and fatty acid composition, has been reported in the young chick after oestradiol administration (Pearce & Balnave, 1975). In fact, one of the minor breakdown products of aflatoxin B_1 in the chick is aflatoxin M_1 (Patterson, 1973) which is known to have a low oestrogenic potency (Kyrein, 1974). These two factors could explain the increased lipogenic enzyme activity at 15 d in Expt 5 and the delay in the development of a fatty liver in chicks fed aflatoxin B_1.

Aflatoxin significantly altered liver fatty acid composition. Veen (1967) has reported similar changes in the liver fatty acid pattern of young piglets given aflatoxin. The inclusion of dietary biotin in Expt 3 substantially reduced the extent of these changes but this response was not observed in the presence of 18 mg retinol/kg. Because of this observation and the reported involvement of biotin in a number of fatty liver conditions (Marchetti & Puddu, 1964; Marchetti *et al.* 1966; Payne *et al.* 1974; Whitehead *et al.* 1974; Balnave *et al.* 1977), liver biotin levels were determined in the subsequent experiment. These showed that the reductions in the liver biotin concentrations observed as a result of feeding aflatoxin were a reflection of the increased lipid content of the liver and that, apart from the situation where no retinol supplement was added to the diet, the amount of biotin on a cellular basis was not decreased. Here again a different response was observed with avitaminotic A chickens. It is also interesting to note that the changes in liver weight, liver lipid and liver fatty acid composition resulting from biotin supplementation of the aflatoxin-contaminated diet in Expt 3 are similar to those observed when biotin-deficient chicks are injected with the vitamin (Balnave, 1966).

The nucleic acid concentrations of chicken livers were substantially influenced by both aflatoxin and retinol. The aflatoxin-induced reduction in RNA concentration is in agreement with the findings of Carnaghan *et al.* (1966) and Tung *et al.* (1972). These authors did not observe a significant reduction in liver DNA concentration. The aflatoxin-induced reduction in liver RNA:DNA has been reported previously (Carnaghan *et al.* 1966; Tung *et al.* 1972) and is consistent with the suggestions that aflatoxin impairs protein synthesis.

The results of these studies indicate that the response of chickens to aflatoxin B_1 can be considerably influenced by the vitamin status of the diet. It is thus important to consider possible interactions of aflatoxin with other dietary components when attempting to correlate aflatoxin intake with metabolic disorders. Delineating these interactions may also be important in human nutrition since those regions of the world with climates most conducive to fungal proliferation are often those areas where malnutrition is prevalent.

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