THE CLINICAL BIOCHEMISTRY OF CHICKS WITH AFLATOXICOSIS: SOME EFFECTS OF SUPPLEMENTARY CHOLINE, FOLATE, THREONINE, LYSINE AND LYSINE PLUS ARGININE

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THE CLINICAL BIOCHEMISTRY OF CHICKS WITH AFLATOXICOSIS: SOME EFFECTS OF SUPPLEMENTARY CHOLINE, FOLATE, THREONINE, LYSINE AND LYSINE PLUS ARGININE

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FOR MY MOTHER

who might have got an MA instead of ME

ABSTRACT

Aflatoxin (2.5ug/g diet) was fed to broiler chicks for 24-26 days in five separate feeding trials in which the effects of supplementary choline, folate, threonine, lysine, and lysine plus arginine were examined. Weight gain, feed intake, feed conversion and hepatic lipid responded in a manner typical for aflatoxicosis. Plasma concentrations of LDH, taurine, tyrosine, phenylalanine, arginine, ornithine, citrulline, glutamine, ammonia and perhaps BUN were increased in response to aflatoxin, while plasma levels of threonine, lysine, total protein, albumin/globulin (A/G) ratio, uric acid, cholesterol, calcium, inorganic phosphate, total iron, total iron binding capacity, and percent saturated transferrin, decreased.

Intraperitoneal (IP) administration of choline, but not dietary choline, moderated the influence of aflatoxin on the majority of the biochemical parameters.

Lysine supplementation improved the performance of chicks with aflatoxicosis, while threonine had a negative effect. This may be related to ornithine detoxification of aflatoxin through the opposing effects of these two amino acids on the activity of the enzyme arginase, which catalyzes the conversion of arginine to ornithine and urea.

Plasma lysine concentration varies considerably as a result of genetic differences in lysine metabolism. Data indicates that

chicks with high plasma lysine concentration are more resistant to aflatoxicosis than chicks with low plasma concentrations of lysine.

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ABBREVIATIONS

AAA..................aromatic amino acid

A/G..................albumin/globulin ratio

Ala..................alanine

Alb..................albumin

ALT..................alanine aminotransferase

AMN..................ammonia

AP..................alkaline phosphatase

App..................appendix

Asn..................asparagine

Asp..................aspartic acid

AST..................aspartate aminotransferase

Arg..................arginine

BCAA..................branched chain amino acid

BUN..................blood urea nitrogen

C..................celseus

Ca..................calcium

Chol..................cholesterol

Cit..................citrulline

Cth..................Cystathionine

Cys..................cysteine

dwb..................dry weight basis

Eth..................ethanolamine

Fig..................figure

g.................gram

GAL...............galactosyltransferase

Gln...............glutamine

GPr...............globulin protein

Glu...............glutamic acid

Gluc..............glucose

Gly...............glycine

Hcy...............homocysteine

His...............histidine

HOL...............hydroxylysine

HOP...............hydroxyproline

HPLC..............high pressure liquid chromatography

Ile...............isoleucine

IP................intraperitoneal

LDH...............lactate dehydrogenase

Leu...............leucine

Lys...............lysine

Met...............methionine

NSP...............ninhydrin positive substance

NRC...............national research council

Orn...............ornithine

P.................inorganic phosphate

PCV...............packed cell volume

Phe...............phenylalanine

Pro...............proline

SAL...............sialyltransferase

Ser...............serine

SFe...............percent saturated transferin

Tau...............taurine

TB................total bilirubin

TFe...............total iron

TIBC..............total iron binding capacity

Thr...............threonine

TLC...............thin layer chromatography

TPr...............total protein

Tri...............triglyceride

Trp...............tryptophan

Tyr...............tyrosine

U.................unit

UA................uric acid

Val...............valine

CHAPTER I

INTRODUCTION

In this chapter, general aspects of toxin producing moulds are reviewed. An historical account of aflatoxicosis is included, with a discussion of the chemical structure of aflatoxin and current knowledge of aflatoxin metabolism. The preliminary work which led to the final choice of this thesis topic is also presented. Additional reviews which appear before chapters III, VI, and V contain more detailed information of particular relevance to the subject matter of the chapter in question.

Toxin producing moulds

Mycotoxins are poisonous substances produced in a substrate as the result of the growth and metabolism of moulds. Production of these toxic metabolites is dependent on the presence of a toxin producing mould in combination with the appropriate temperature, oxygen concentration, moisture level, and substrate. Optimal conditions for mould growth are not necessarily optimal conditions for toxin production (1). It is estimated that 30-40% of all moulds are capable of producing mycotoxins under certain conditions. Crops may be infested by moulds prior to harvest, usually after a plants natural defense mechanisms have been weakened by insect infestation or harsh weather conditions. Fungal infections also occur post-harvest, and are a result of inadequate dehydration or improper storage and handling. Control of moisture and temperature are particularly critical in this respect (1). A given toxin may be produced by a single or many different mould species and the different mycotoxins vary considerably in chemical form and properties. Although the toxin producing moulds are not restricted to any single group of moulds, the genera in which they occur most frequently are Aspergillus, Penicillium, and Fusarium. A group of closely related metabolites produced by strains of Aspergillus flavus and Aspergillus parasiticus are known collectively as aflatoxin. These fungi are ubiquitous, and thus the potential for the outgrowth of aflatoxin producing fungi in foodstuffs and animal feeds is widespread. Corn and peanuts are particularly susceptible to aflatoxin contamination and both are major ingredients of animal feeds. Aflatoxin is not only the most prevalent, but also the most toxic and carcinogenic mycotoxin known. Aflatoxin contamination of grains is normally associated with warm climates although the lower limiting temperature for growth of A. flavus is 12C (1). Hence, Newfoundland is not generally thought of as a region in which aflatoxin contamination should be significant. However since all of the grain utilized in Newfoundland is imported, the environmental conditions encountered during transportation and storage, as well as the quality of the original product may be more relevant to the levels of aflatoxin or other mycotoxins in Newfoundland feeds. A snowballing effect has been demonstrated in which there is an accumulative increase in the content of mycotoxins with each step of their journey from the field to the feed trough (grain elevator, truck, shipping car, warehouse, barn loft etc.) (2). This suggests that the greater the geographical separation between the sites of production and consumption of a given product, the greater the expectation of mycotoxin contamination. Even if a product contains negligible concentrations of a toxin at the site of production, the levels may have increased to biologically important concentrations by the time the feed is consumed in Newfoundland. In addition there is an absence of routine testing of feeds for aflatoxin or other mycotoxins in Newfoundland, and the symptoms of aflatoxicosis are non-specific and thus not easily diagnosed. Therefore, Newfoundland appears to be a more likely target for aflatoxicosis then might be predicted at first glance.

Feed survey

Before chosing a mycotoxin to study intensively through feeding trials, a preliminary survey of mycotoxins occurring in animal feeds in Newfoundland was undertaken. One hundred and eleven samples of feed including rations for poultry, cattle, and swine from accross the province were analyzed for the presence of fourteen different mycotoxins (zearalenone, sterigmatocystin, roridin A, T-2 toxin, penicillic acid, patulin, diacetoxyscirpenol, verrucarin A, ochratoxin A, citrinin, and the four aflatoxins B-1, B-2, G-1, and G-2). Thirty-seven milk samples were also analyzed for M-1, a metabolite of aflatoxin B-1 that occurs in the milk of animals fed aflatoxin contaminated feed. Tentative positives for aflatoxin B-1 (often in combination with B-2 and G-1) were obtained from forty percent of the feed samples tested in the initial screening process, but none of these samples were consistently positive throughout all of the subsequent confirmatory tests. Only four of these samples were associated with adverse performance (decreased egg production, feed refusal) which could be attributable to aflatoxicosis. Of the thirty-seven milk samples tested one positive M-1 was obtained. Tentative positives for patulin, verrucarin A, and penicillic acid were obtained in 6%, 8%, and 17% of the feed samples respectively, while less than 5% of the samples showed tentative positives for T-2, zearalenone, stergmatocytin, ochratoxin and citrinin. Roridin A and diacetoxyscirpenol were negative in all samples. Positive identification of these toxins by comparison of sample extracts with mycotoxin standards subjected to thin layer chromatography was difficult because of the large number of standards to be run with each sample. In addition, the large number of different components in the feed extracts (due to the broad specificity of the extraction procedure) influenced the colour and Rf of different components of the extract on the chromatograms, making identification by comparison with individual standards unreliable. Towards the end of this survey it became apparent that the TLC methods of screening for multiple-mycotoxins are inadequate to permit the confirmation of specific mycotoxins. For large scale surveys, TLC methods have been rapidly replaced by computerized HPLC systems which can store the HPLC profile of mycotoxin standards for future reference. Using this system, sample extracts are simply injected into the HPLC unit, and all mycotoxins present are identified and quantitated automatically. Because the object of the project sponsored by MRC was to determine the influence of diet on avian mycotoxicosis, an expensive change in the methodology of the survey could not be justified, and the research was redirected toward feeding studies utilizing aflatoxin and poultry.

History of aflatoxin induced disease

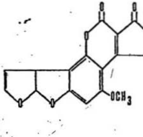
Aflatoxicosis received world wide attention in 1960 when *A. flavus* contaminated peanut meal was implicated as the cause of the mysterious 'turkey X disease' which was responsible for the deaths of at least 6,000 chicks and 12,000 ducklings in addition to 100,000 turkeys in the United Kingdom. The disease was characterized by sudden loss of appetite, subcutaneous haemorrhages and a high and rapid mortality in young birds. At postmortem the livers were pale, fatty, and showed extensive biliary proliferation. Partridge and pheasant poults together with cattle, pigs and sheep were also affected. Almost simultaneously, many trout bred in commercial hatcheries in the United States developed hepatomas and this outbreak was eventually attributed to the same fungal toxin (3). Since 1960, numerous studies have been conducted to determine the chemical nature of these *A. flavus* toxins and the clinical problems encountered as a result of their consumption. Four major chemically related aflatoxins designated B1, G1, B2, and G2 commonly occur together in contaminated feeds and feedstuffs. The proportions in which these metabolites are produced may vary considerably depending on environmental conditions, particularly temperature. Aflatoxin produced in this laboratory consisted of 88% B1, but the proportion of G1 is reported to be increased substantially at lower temperatures (1).

Structure and metabolism of aflatoxin

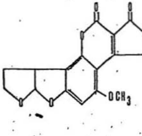
The aflatoxins fluoresce strongly in ultraviolet light (ca

365nm); B1 and B2 produce a blue fluorescence whereas G1 and G2 produce a green fluorescence. The structures of these compounds are shown in Fig.1. Because aflatoxin B1 is the most prevalent as well as the most potent of the aflatoxins, the metabolites of aflatoxin B1 have been studied most intensively, and will be reviewed below. B2, G1, and G2 undergo most of the same reactions to produce the corresponding B2, G1, or G2 derivative (3). In general the detoxification of xenobiotics by the liver results in the formation of more polar products which may or may not be subsequently conjugated with amino acids, glucuronic acids, sulfate or bile acids as an aid to their excretion. The aflatoxin molecule lends itself to biodegradation in at least 6 ways (3), as shown in Fig. 2. Reactions involving the intact molecule have been confirmed by the isolation of the metabolites which are cited as examples.

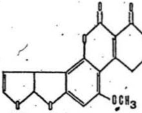
The predominant biotransformations vary between animals of different species, a fact which partially explains the wide variation in species susceptibility to aflatoxicosis. A number of dietary factors have been found to influence the metabolism, and thus the toxicity of a given dose of aflatoxin. In the following chapters, the influence of specific dietary factors that are relevant to the corresponding nutritional study will be discussed in more detail. The feeding trials examine the effects of supplemental choline, folate, threonine, lysine, and lysine plus arginine on chicks with aflatoxicosis and are referred to as feeding trials 1, 2, 3, 4, and 5, respectively.



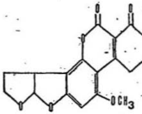
AFLATOXIN B1



AFLATOXIN B2



AFLATOXIN G1



AFLATOXIN G2

Fig. 1. Structures of Aflatoxin B1, B2, G1, and G2.

Fig. 2. Metabolism of Aflatoxin B1.

a) Reductive or hydrolytic attack on the 2,3 double bond. Examples include the 2,3-dihydroxy, the 2-hydroxy (also known as B2a or hemiacetal) and 2,3-epoxide derivatives. The 2,3-epoxide is assumed to be an important reactive intermediate and has never been isolated although there is good evidence of its formation.

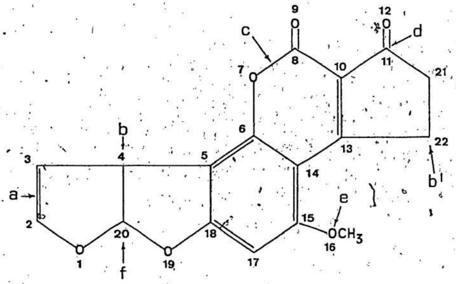
b, b') Hydroxylation at one or more points in the molecule. Both aflatoxin M1 and Q1 are examples of hydroxylated derivatives (4-hydroxy and 22-hydroxy respectively).

c) Hydrolytic fission of the coumarin lactone.

d) Cyclopentenone reduction. Aflatoxin Ro, also referred to as aflatoxicol, results from reduction of the 11,12 double bond.

e) O-demethylation of the methoxy-coumarin structure. Aflatoxin P1 refers to the phenolic compound derived in this manner.

f) Opening of the bisfuranoid structure.


9
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CHAPTER II

MATERIALS AND METHODS

Feed collection and storage

Farmers and feed manufacturers in Newfoundland were requested to submit samples of feedstuffs for analysis of mycotoxins from June 1979 to August 1980. Initial contact was made by telephone, from a list provided by Dr. A. Smith (provincial veterinarian). Packages containing an explanatory letter, sample submission forms, plastic bags for sample submission and return address labels with guarenteed postage were mailed to all individuals who responded favourable to the initial telephone communication. The respondents included three feed manufacturers and 144 farmers involved with swine, cattle, sheep, horses, and poultry. The regular submission of unbias samples was requested in addition to bias samples. Bias samples were defined as feeds associated with feed refusal or undesirable animal performance and/or feed having a mouldy or otherwise unusual appearance or odour. A total of 111 feed samples were submitted over the entire 14 month period, and 35% of these were bias samples. Milk samples were obtained from five separate commercial dairies and included samples of raw, whole, 1%, 2%, skim, and chocolate milk. Milk was obtained over a period of 3 months starting in November 1979. All feed and milk samples were stored in sealed bags or bottles at 30C.

Mycotoxin extraction

Feed samples were extracted by the procedure for the extraction of mycotoxins described by Patterson and Roberts (4). This procedure produced two separate extracts from each feed sample, one containing basic mycotoxins (ochratoxin, and citrinin) and a second containing acidic mycotoxins (aflatoxin, zearalenone, roridin A, T-2 toxin, penicillic acid, patulin, verrucarin A, stermatocystin, and diacetoxyscirpenol).

Milk samples were extracted by the aflatoxin M1 procedure of Stubblefield (5).

Thin layer chromatography

Aflatoxin M1 extracts of the milk samples were evaporated to dryness under nitrogen and redissolved in 100ul chloroform. Twenty ul aliquots were developed on one dimensional precoated silica gel TLC plates (Macherey-Nagel, Germany; SIL G-25HR) in isopropanol/acetone/chloroform (2:10:88) and visualized under longwave UV light. Positive samples were confirmed using two dimensional TLC with the multiple solvent systems recomended for aflatoxin by Patterson and Roberts (4).

All feed extracts were initially screened on one dimensional silica gel TLC plates. An aliquot containing the various reference standards was also applied to each plate. Extracts were dried under nitrogen and redissolved in 100ul chloroform. Twenty ul of the extract containing the basic mycotoxins were developed in toluene/ethyl acetate/90% formic acid (60:30:10) and visualized under longwave UV light. Twenty ul of the extract containing acidic mycotoxins were developed in chloroform/acetone (90:10) and followed by the visualization treatments recommended by Patterson and Roberts (4) for the general screen. Two dimensional TLC, combined with chromatogenic derivative formation were used to confirm the presence of specific mycotoxins in a sample extract, using the solvent system and visualization sequence recommended by Patterson and Roberts (4) for the specific toxin.

Aflatoxin production

Aflatoxin used in this study was produced by Aspergillus parasiticus NRRL 2999 on sterile polished rice by the method of Shotwell et al. (6) as modified by West et al. (7). However, to prevent adhesion of the rice, the sterile rice and water were combined (2:1, respectively) at 100C and the mixture was allowed to steam under reduced heat until the water was absorbed (ca 20 min). After cooling, the rice was aseptically transfered into 250 ml Erlenmeyer flasks, inoculated and incubated under the conditions described by West et al. The mouldy rice was heated at 100C for 3 min. to inactivate the fungus and then dried and ground to a fine powder using a ballmill. The powder was analyzed for total aflatoxin content by the spectrophotometric method of Nabney and Nesbitt (8) as modified by Wiseman et al. (9). The rice powder contained aflatoxin in the following ratio: B1:G1:B2:G2 (88:9:2:1). Rice powder was added to the basal ration to obtain 2.5ug aflatoxin /g of feed. Rice powder added to the feed never exceeded 0.2% of the total diet.

Animal husbandry and necropsy

Day-old chicks (cockerels, Hubbard/Hubbard) were reared in wire-floored brooder batteries (31cm X 50cm X 23cm) with constant illumination at a light intensity of 56 footcandles. Water was available from separate containers for each cage of chicks from the day of hatch until termination of the experiment. The lysine plus arginine feeding trial terminated after 26[a] days while the other feeding trials concluded after 24 days. The basal ration consisted of a commercial broiler starter ration free of all medications. The formulation and amino acid composition of the rations are listed in Tables I and II, respectively. Four separate 3x3 factorial experiments were completed in which the influence of added supplements of choline, folate, threonine or lysine on aflatoxicosis in chicks was examined. The particular levels where chosen to provide supplementation substantially above the NRC requirement for broiler chicks and that provided by common premixes used by industry (Hoffman-LaRoche) and universities (UGA), yet with care to avoid levels which could produce toxicity. The levels of threonine, lysine and arginine supplements were also chosen with reference to information provided in the reports of Austic (79,80), which discuss the effects of supplementation of these amino acids to broiler chicks at a variety of levels and

[a] Termination was delayed in this feeding trial because freeze clamping equipment required for liver preparation by a co-worker for a separate project was not available on day 24.

Table I. Composition of the basal ration.

Ingredient	Composition[a]
Ground yellow corn	57.46
Soybean oil meal (dehulled)	30.84
Poultry by-product meal	4.9
Fat (vegetable)	2.98
Ground limestone	1.15
Dicalcium phosphate	1.74
Salt	0.04
Methionine (DL)	0.15
Trace mineral mix[b]	0.05
Vitamin premix[c]	0.25
Protein	23.0
Metabolizable energy[d]	3.120 MJ/Kg
Calcium[d]	1.09
Phosphorus (available)[d]	0.51

[a] Percentage unless indicated otherwise.

[b] Supersweet Feeds Mineral Premix (International Multifoods Corp., Minneapolis, Minnisota) provided the following minerals (mg/kg of diet): manganese 55, zinc 80, iron 80, copper 11, iodine 0.38.

[c] Vitamin premix provided the following amounts of vitamins per kg of diet: vitamin A, 4,400 IU; vitamin D_3, 880 ICU (International chicken Units, 0.025 ug of cholecalciferol = 1 ICU); vitamin E, 11 IU; riboflavin, 4.4 mg; calcium pantothenate, 9.6 mg; nicotinic acid, 44 mg; choline chloride 220 mg; vitamin B_{12}, 6.6 ug; pyridoxine hydrochloride, 2.2mg; menadione sodium bisulfite 3.49 mg; folic acid, 0.55 mg; D-biotin, 0.11 mg; thiamine mononitrate, 2.2 mg; ethoxyquin, 125 mg. Choline or folate were deleted from the premix for the corresponding studies. Vitamins were supplied by Hoffman-La Roche Inc. (Chemical Division, Nutley, New Jersey).

[d] Calculated values (National Research Council, 1977, Nutrient Requirements of Poultry, 7th edition, National Academy of Science, Washington D.C.).

Table 11. Content of amino acids in the basal diets for the choline, folate, lysine, threonine and lysine + arginine feeding trials.

Amino acid	Choline/Folate µmol/g of diet	Choline/Folate NRC (%)	Lysine µmol/g of diet	Lysine NRC (%)	Threonine µmol/g of diet	Threonine NRC(%)	Lysine + Arginine µmol/g of diet	Lysine + Arginine NRC (%)
Alanine	136±6.8	NR [c]	132±9.7	NR	139±3.8	NR	125	NR
Ammonia	263±14	NR	264±19	NR	262±8.8	NR	268	NR
Arginine	78.2±4.6	95	78.0±6.9	94	78.4±2.2	95	75.5	91
Aspartic Acid	166±7.6	NR	161±14	NR	170±1.2	NR	169	NR
Glycine	149±8.8	147[d]	143±11	143[d]	155±6.6	150[d]	135	139[d]
Glutamic Acid	273±14	NR	271±21	NR	274±6.7	NR	268	NR
Half cystine	27.2±5.6	77[de]	14.2±1.7	54[de]	14.1±3.6	52[de]	62.3	126[d]
Histidine	36.8±1.7	163	36.3±2.9	161	37.2±0.57	165	35.2	156
Hydroxyproline	24.9±3.0	NR	21.2±3.3	NR	28.6±2.7	NR	7.82	NR
Isoleucine	60.9±9.2	99	62.5±11	102	5.87±7.4	96	57.1	93
Leucine	138±8.8	134	137±12	133	138±5.6	134	138	133
Lysine	84.1±4.6	103	83.4±6.5	102	84.7±2.7	103	72.5	88
Methionine	26.1±3.0	78[e]	23.3±3.3	67[e]	20.8±0.95	62[e]	28.9	86
Phenylalanine	60.1±4.2	138	61.0±5.1	140	59.2±3.2	136	62.2	142
Proline	121±6.1	NR	121±9.3	NR	121±2.8	NR	128	NR
Serine	103±3.8	147[d]	102±3.7	143[d]	103±3.9	150[d]	102	139[d]
Threonine	74.0±3.4	118	67.2±4.7	107	80.7±2.1	128	69	109
Tyrosine	27.5±2.1	111[d]	27.3±2.0	112[d]	27.7±2.1	110[d]	35.4	124[d]
Valine	79.5±12	113	81.4±14	116	77.6±9.1	110	75.3	107

[a]Means ± standard deviations (for the choline and folate experiments the values are from nine analyses; for the lysine experiment from five analyses, for the threonine experiment from duplicate analyses; for the lysine + arginine experiment from duplicate analyses).
[b]Percentage of the NRC dietary requirement (1977).
[c]NR = no NRC requirement.
[d]Glycine + serine or half cystine + methionine or phenylalanine + tyrosine.
[e]Analytical values are low, when compared to the calculated contents from feed composition. Calculated contents (% NRC) methionine = 103%, methionine + cystine = 97%.

combinations.

In each experiment, one-third of the chicks received a diet containing 2.5ug aflatoxin/g of feed. The remaining chickens provided two sets of controls. One group of controls was pair-fed to the feed intake of the birds that received aflatoxin, while the remaining chicks were allowed to consume feed <u>ad libitum</u>. Each of the three primary treatments was subdivided into three groups in which the concentration or mode of administration of the supplemented vitamin or amino acid was varied. In the experiment with choline, the chicks recieved either the basal ration without the addition of choline, which supplied 106% of the NRC requirement of choline, or else equivalent dosages of choline via diet or intraperitoneal (IP) injection to achieve 175% of the NRC requirement of choline. In the folate experiment, the basal ration provided 244% of the NRC requirement of folate and was supplemented to produce two additional diets containing folate at concentrations of 344 and 644% of the NRC requirement. Similarly, in the threonine experiment the basal diet provided 128% of the NRC requirement, and was supplemented to produce two additional diets containing 155 and 179%. In the lysine experiment, the basal ration containing 102% of the NRC requirement was supplemented to 122 and 146%. A fifth, 4x3 factorial experiment was completed in which the influence of concurrent dietary administrations of lysine and arginine were studied. Each of the three primary groups (aflatoxin, pair-fed, and ad libitum controls) were subdivided into four groups in which the concentrations and proportions of lysine and arginine were varied. The basal ration provided 102% and 94% of the NRC requirements of lysine and arginine, respectively. This ration was supplemented to produce three additional diets containing lysine and arginine in the following percentages of the NRC requirement: 102% lysine and 122% arginine, 122% lysine and 122% arginine, and 146% lysine combined with 122% arginine. In all five experiments, each experimental treatment was given to four replicate pens of chicks from the day after hatch. The pens initially contained 6 chicks each and were reduced to 5 chicks each on the seventh day.[a] The experimental treatments were assigned in a completely randomized design. At the termination of the feeding trials, a 5 ml aliquot of blood was obtained from each chick by cardiac puncture using a syringe containinng 100 U of sodium heparin. After bleeding, the chicks were killed by cervical dislocation. Livers were excised. All samples were stored at -30C until analyzed with the exception of the livers from feeding trial 5, which were stored at -60C after freeze clamping in liquid nitrogen.

Animal performance and biochemical analyses

Feed intake and mortality were recorded daily, while weight gain was recorded daily for the first two experiments, but at three day intervals for the remaining experiments. The following biochemical

[a]Six chicks were initially provided/cage so that chicks lost due to congenital abnormalities or early mortality could be replaced.

parameters were measured at the termination of the experiment: calcium (10), inorganic phosphate (11), total iron, total iron-binding capacity (TIBC) and transferrin iron (12), glucose (13), triglyceride-glycerol (14), glycerol (bacterial lipase modification of procedure 15), cholesterol (16), protein (17), albumin (18), blood urea nitrogen (BUN,19), uric acid (20), bilirubin (21), creatine (22), alkaline phosphatase (AP, 23), lactate dehydrogenase (LDH, 24), asparate aminotransferase (AST, 25), alanine aminotransferase (ALT, 26), sialytransferase and galactosyltransferase (SAL, GAL, 27) and hepatic lipid (28). Hepatic moisture was determined by drying for 24h at 110C. LDH isozyme patterns in various tissues, plasma and packed blood cells were analyzed by polyacrylamide gel electrophoresis and stained for enzyme activity (29). The plasma concentrations of free-amino acids and ninhydrin positive substances (NPS) were determined using the supernatants from mixtures of 0.8 ml of plasma and 0.8 ml of 2.5% sulfosalicylic acid in lithium citrate buffer (0.15N pH2.2) using a Beckman 121-MB amino acid analyzer and employing a physiological fluid program. The amino acid profiles of feed were also determined using the Beckman 121-MB from 6N hydrochloric acid digestions (_in vacuo_, 110C, 24h). The digestions for methionine and cysteine employed a hydrogen bromide/performic acid oxidation prior to the hydrochloric acid digestions (30), while the analyses for tyrosine contained 0.1% thioglycolic acid and 0.05% phenol in the hydrochloric acid digestions (31). Sigma Kit No. 565 (St. Louis, Missouri) was used for the iron profiles. Cholesterol, triglyceride-glycerol and ALT evaluations were assayed with Abbott Laboratory kits (South Pasadena, Californa). Glycerol profiles were determined with Boehringer Mannheim Kit No. 148270 (Mannheim, West Germany). Except for SAL and GAL, the remaining blood analyses were performed using the Technicon SAM 12/60 autoanalyzer.

Statistical analyses

All performance and biochemical results within a given feeding trial were statistically evaluated by implementing SAS software packages (Statistical Analysis System Instituter Inc., Cary, North Carolina) within an IBM 370/158 or AMDAHL computer system. The statistical procedures included evaluation for the occurance of interaction in the major effects as well as analysis of variance, Duncan's multiple range test and the Waller-Duncan K-ratio t-test. Statements of significance are based on $P \leq 0.05$.

Data presented in chapter III are averages of the values (mean and standard deviation) obtained from the chicks receiving the basal ration (no supplementation) in each of the individual feeding trials.

CHAPTER III

EFFECTS OF AFLATOXIN

Performance

Before one can study the biochemical effects of dietary supplements on chicks with aflatoxicosis, one must first describe the biochemical changes produced by aflatoxin. This is also necessary in order to demonstrate that a typical aflatoxin lesion has been produced in a given experimental trial. Anorexia, impaired performance and accumulation of hepatic lipid are widely recognized as typical responses to ingested aflatoxin in poultry and most other species of animals (32). The data presented in this chapter, unless otherwise specified, are the combined values obtained from the aflatoxin and control groups receiving the basal ration in the five separate feeding trials. The actual numerical values obtained in the individual experiments from which these figures were derived, are shown in App. 1-25. Fig. 3 shows the effect of aflatoxin on performance of chicks after 24 days of experimental treatment. Weight gain was significantly reduced in the pair fed controls, and further reduced by aflatoxin. This indicates the presence of both an anorexic and a toxic component of this response. The toxic component (decreased weight gain over and above the decrease attributable to the anorexic effects of aflatoxin) was significantly different in only two (folate, and lysine) out of the five feeding trials, and will be discussed in detail in chapter VI. A decreased rate of weight gain (Fig. 4) was apparent by the 6-8th day in the birds receiving aflatoxin, while a decreased feed intake was observed by the 10th day (Fig. 5).

Fig. 3. Effect of aflatoxin on the performance of chicks. Values are expressed as a percentage of the ad libitum control and are averages of the data from the aflatoxin and control groups which received the basal ration (no supplementation) in the five separate feeding trials.

Weight gain, feed consumption, and feed conversion (feed consumption/weight gain) are impaired by aflatoxin. Weight gain is significantly reduced compared to the ad libitum controls, and further reduced compared to the pair-fed controls. This reduction was significant in some feeding trials but not in others and is discussed in detail in chapter VI.

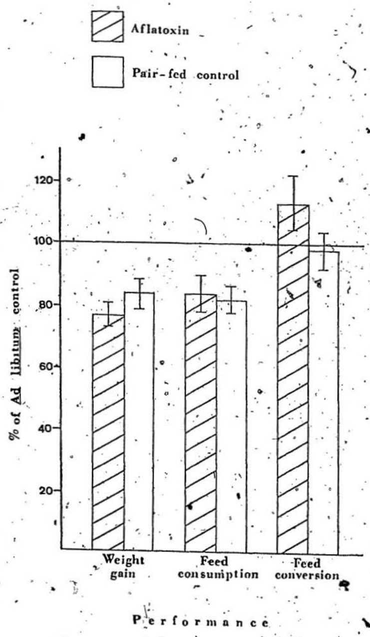
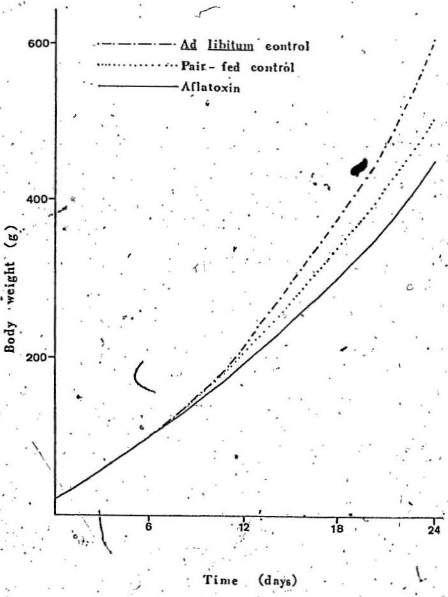
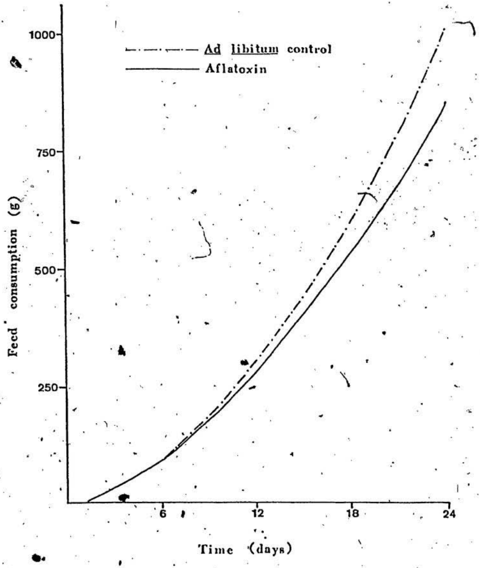

Aflatoxin
Pair-fed control
120
100
80
60
40
20
% of Ad libitum control
Weight gain
Feed consumption
Feed conversion
Performance

Fig. 4. Daily weight gain of chicks with aflatoxicosis. Values are averages of the data from the aflatoxin and control groups which received the basal ration (no supplementation) in the five separate feeding trials.



Ad libitum control
Pair - fed control
Aflatoxin
600
400
200
Body weight (g)
6
12
18
24
Time (days)

Fig. 5. Daily feed consumption of chicks with aflatoxicosis. Values are averages of the data from the aflatoxin and control group which received the basal ration (no supplementation) in the five separate feeding trials.


1000
750
500
250
Feed consumption (g)
Ad libitum control
Aflatoxin
6
12
18
24
Time (days)

Hepatic changes

Hepatic weight, moisture, and lipid content are shown in Fig. 6. The % lipid/g liver can be seen to have been increased by an average of 80%, with an increase in total weight but without an increase in % moisture. Because the liver is considered to be the primary target tissue of aflatoxin, the plasma concentration of a series of hepatic enzymes were measured in plasma as an indication of hepatic damage (feeding trials 1-4 only). Fig. 7 shows that of the enzymes measured, LDH alone was found to increase significantly. An elevation in the activity of LDH of 80-100% was consistent throughout all four experiments. The distribution of aflatoxin-derived radioactivity in the rat, mink, rhesus monkey and swine shows that liver, and to a lesser degree kidney and heart, are the most susceptible tissues for macromolecular binding (33), while in poultry, particularly in broiler chicks, the metabolites are more widely dispersed in all body tissues (34,35). This suggests that the hepatotoxicity of aflatoxin may be less in poultry than in certain other species. Garlich et al. have reported an increase in AP in laying hens with 20ug/g of dietary aflatoxin (36). In duckings, Brown and Abrams found a slight increase in plasma levels AP, AST, and ALT after receiving 0.5ug/g of dietary aflatoxin for 4 weeks. The elevations progressed to large increases in AST and ALT at 8 weeks, while LDH was markedly elevated at both time intervals (37). Aflatoxin has previously been shown to cause increases in plasma bilirubin, ALT, AST, and cholesterol in goats, cattle, rabbits (38), which are all indications of liver damage. In this

Fig. 6. Effect of aflatoxicosis on hepatic weight, percent moisture, and percent lipid. Values are expressed as a percentage of the *ad libitum* control and are averages of the data from the aflatoxin and control groups which received the basal ration (no supplementation) in the five separate feeding trials, with the exception of hepatic weight which represents data from feeding trials 1-4 only. Livers from feeding trial 5 were freeze clamped, and total liver weight was not measured.

Hepatic weight and percent lipid are increased by aflatoxicosis.

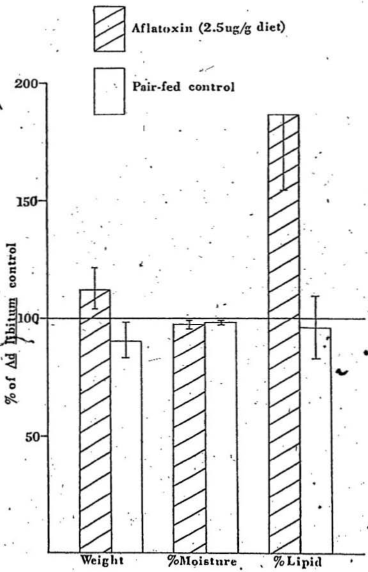
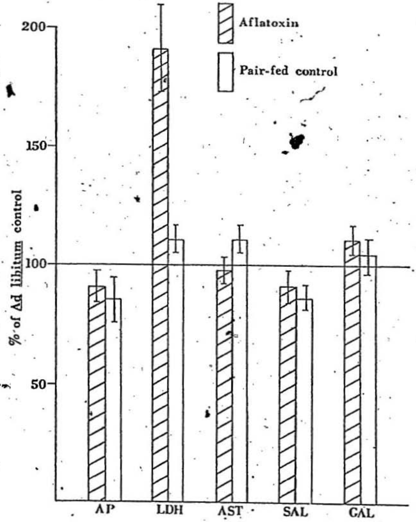

Aflatoxin (2.5ug/g diet)
Pair-fed control
200
150
100
50
% of Ad libitum control
Weight
%Moisture
%Lipid

Fig. 7. Effect of aflatoxicosis on plasma levels of selected hepatic enzymes. Values are expressed as a percentage of the *ad libitum* control and are averages of the data from the aflatoxin and control chicks which received the basal ration (no supplementation) from three or more separate feeding trials as detailed below; Alkaline phosphatase, lactate dehydrogenase, and aspartate aminotransferase are averages from feeding trials 1-4. Sailyltransferase and Galactosyltransferase are averages of data from feeding trials 1-3 only. The analyses were not completed on plasma from the corresponding feeding trials omitted from the calculations, due to the high cost of the assays relative to the amount of new information one would expect the assays to provide.

Only LDH was increased significantly by aflatoxicosis.


Aflatoxin
Pair-fed control
200
150
100
50
% of Ad libitum control
AP
LDH
AST
SAL
GAL

series of experiments with broiler chicks, the failure of aflatoxin to induce plasma increases in the other hepatic enzymes, suggests that the plasma LDH probably originates from some tissue other than the liver. LDH activities (Table III) and zymograms (Fig. 8) from various tissues, packed blood cells, and plasma suggest that the elevated LDH activity in plasma originated from hemocytes and not from hepatocytes. This implication is consistent with the unchanged plasma levels of the other hepatic enzymes, as well as a previous report that the fragility of red blood cells increases with aflatoxicosis (39,40). The numbers of the various types of leucocytes, which contain about 50 fold higher level of LDH than erythrocyes, or thrombocytes (41) are markedly altered by aflatoxicosis and thus may contribute to the rise in plasma LDH.

Clinical parameters

To establish a clinical picture of the aflatoxin lesion, a series of standard clinical analyses were measured in plasma as shown in Fig. 9. The analyses selected were those which can be obtained rapidly by a single administration of a plasma sample to a standard clinical autoanalyzer (available in the clinical laboratory of any hospital). It was hoped that a distinct pattern or "biochemical fingerprint" could be determined for aflatoxicosis which could them be used to distinguish aflatoxicosis from other syndromes in commercial poultry operations. This fingerprint would also provide a means of monitoring the effects the dietary supplements on the clinical status of the chicks. BUN, and total bilirubin concentrations in plasma were found to increase in response to aflatoxin, with no change in plasma glucose

Table III. Effect of aflatoxin on LDH activities in various tissues.

Tissue	Aflatoxin	Pair-fed	*Ad libitum*
	Protein (mg/g or ml)		
Liver	80	68	78
R B C	161	154	166
Plasma	12	25	24
	LDH activity (rate/g or ml)		
Liver	129	456	437
R B C	19	25	29
Plasma	0.64	0.46	0.41
	LDH specific activity (rate/mg protein)		
Liver	1.60	6.76	5.64
RBC	0.12	0.17	0.17
Plasma	0.055	0.019	0.017

LDH activities were calculated from linear kinetic data using several incremental aliquots of each sample. Samples were pooled from forty or more individual animals.

Rate = absorbance at 360 nm / minute, or umoles pyruvate reduced / minute.

Fig. 8. Comparison of LDH isozyme patterns from various tissues.

Anaerobic tissues (skeletal muscle, liver) predominantly synthesize the polypeptide chain A which is more efficient under anaerobic conditions, while aerobic tissues (heart, RBC) predominantly synthesize chain B which is more efficient under aerobic conditions. Each isozyme (1–5) is a tetramer, formed from a combination of the two different monomers in all possible combinations, with LDH-1 formed from four B monomers, and LDH-5 from four A monomers.

Fig. 8. shows that liver contains predominantly isozymes 3, 4, and 5, while RBC contains predominantly 2, and 3. Likewise, plasma contains predominantly isozymes 2 and 3. No increase in isozymes 3, 4, and 5 is apparent in the plasma of the birds receiving aflatoxin as would be expected if the increased plasma LDH was of hepatic origin.

Two bands were obtained from the plasma of the birds receiving aflatoxin due to leakage of the sample from the well in which it was applied into an adjacent well at the origin.

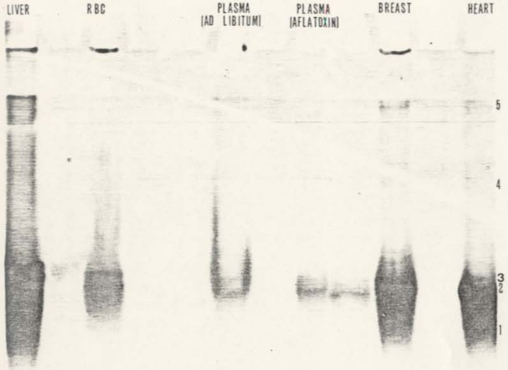
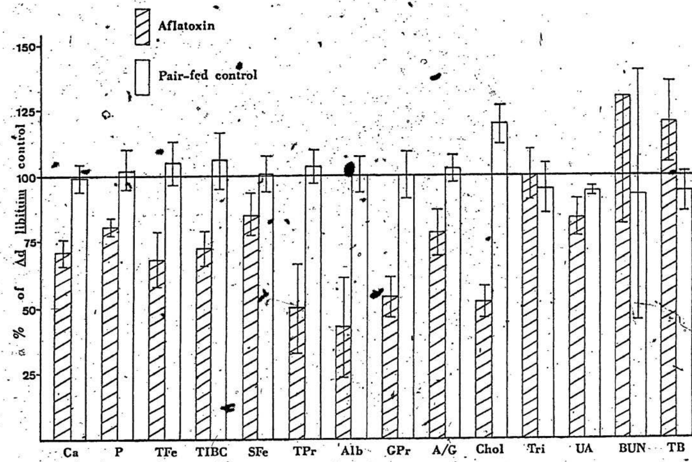

LIVER
RBC
PLASMA (AD LIBITUM)
PLASMA (AFLATOXIN)
BREAST
HEART
5
4
3
2
1

Fig. 9. Effect of aflatoxicosis on the clinical biochemistry of chicks. Values are expressed as a percentage of the ad libitum control and are averages of data from chicks which received the basal ration (no supplementation) in feeding trials 1-4. The screen for clinical parameters was not completed on plasma from feeding trial 5.


Aflatoxin
Pair-fed control
% of Ad libitum control
150
125
100
75
50
25
Ca
P
TFe
TIBC
SFe
TPr
Alb
GPr
A/G
Chol
Tri
UA
BUN
TB

and triglyceride concentration, while the uric acid, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, calcium, inorganic phosphate, total iron, total iron binding capacity, and percent saturated transferin concentrations decreased. BUN and total bilirubin were not present in sufficient concentrations to permit reliable quantitation, although there appeared to be an elevation in these compounds. the findings for calcium, total protein, and glucose are consistent with the responses reported in the literature (37,39,42) while the decrease in total iron and TIBC supports the observation by Lanza et al. that aflatoxin reduces iron absorption (43). The suppression in albumin, globulin, and albumin/globulin ratio has also been reported to occur in duckings and laying hens (37). Other hepatotoxins usually induce an increase in the globulin fraction, and therefore the electrophoretic pattern of plasma proteins obtained with aflatoxin may be of diagnostic significance.

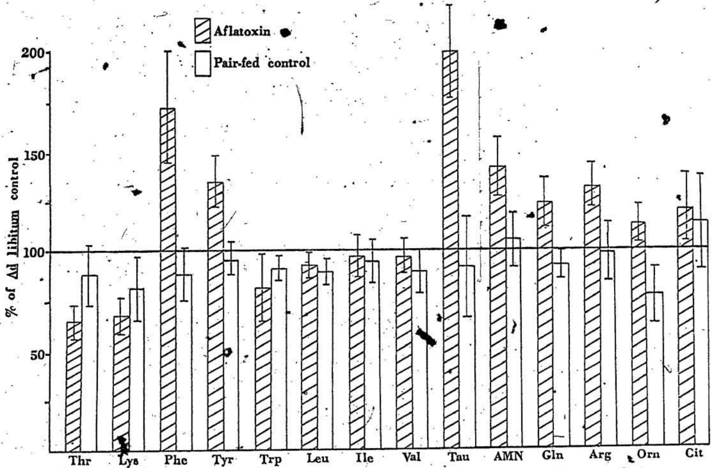
The suppression in plasma concentration of calcium, phosphorus, total protein, albumin, total iron, and cholesterol, but with elevated LDH, is a profile that is typical of malabsorption syndromes (44). Osborne and Hamilton have shown that aflatoxicosis lowers the concentration of bile secreted (45) and decreases the formation of pancreatic lipase (46), thereby impairing the digestion and absorption of lipids, and lipid soluble vitamins. This relationship between aflatoxin and malabsorption will be discussed in more detail in chapter IV.

Plasma free-amino acids

Plasma amino acid response to aflatoxin was also monitored. An earlier study reported that aflatoxin (1.25 or 2.5ug/g of diet), reduced the concentrations of all amino acids in plasma of broiler chicks (Cobb/Cobb). However, the protocol for the previous amino acid determinations involved hydrochloric acid hydrolsis of supernatants obtained from mixtures of sulfosalicylic acid and plasma (47). The acid hydrolysis was necessary since a non-physiological amino acid program was employed. As a result, the values which were obtained included free-amino acids, conjugates, peptides, and proteins not precipitated by sulfosalicylic acid. The amino acid profiles reported in this study were determined using a physiological program, and represent free-amino acid contents. Certain amino acids or amino acid ratios responded to aflatoxin in a remarkably consistent fashion throughout the five experiments, and are shown in Fig. 10. Data for all other amino acids and ninhydrin positive substances showed a non specific or variable response to aflatoxin and are shown in App. 21-25.

Elevated phenylalanine and tyrosine, without an increase in tryptophan, but with a reduction in branched chain amino acids (BCAA) in plasma has been reported by several authors as a result of impaired liver function. This has been rationalized by the fact that the aromatic amino acids (AAA) are metabolized in the liver, while the BCAA are metaboilzed mainly in the muscle. AAA are precursors to various neurotransmitters, and compete with the BCAA for entry through the blood-brain barrier. Changes in the AAA/BCAA ratio have been implicated in the initiation of hepatic coma which is actually

Fig. 10. Effect of aflatoxicosis on the plasma concentrations of selected amino acids. Values are expressed as a percent change of the _ad libitum_ control and are averages of the data from the chicks which received the basal ration (no supplementation) in the five separate feeding trials.


Aflatoxin
Pair-fed control
200
150
100
50
% of Ad libitum control
Thr
Lys
Phe
Tyr
Trp
Leu
Ile
Val
Tau
AMN
Gln
Arg
Orn
Cit

reversible by infusion of BCAA (48,49).

In the data presented in this report, the ratio of the sum of the BCAA, leucine, isoleucine, and valine, to the AAA, phenylalanine, and tyrosine, was reduced from 2.33 and 2.28 in the plasma of the ad libitum and pair fed controls respectively, to 1.27 in the aflatoxin groups. The decreased BCAA/AAA ratio was due primarily to the elevated plasma levels of AAA, and was very likely attributable to an aflatoxin induced impairment of hepatic function. A possible consequence of this altered ratio is that the competitive action of the branched chain amino acids on cerebral uptake of aromatic amino acids would be reduced (50). High levels of phenylalanine in plasma have been shown to raise cerebral levels of phenylalanine sufficiently to competitively inhibit neural tyrosine hydroxylase (51), and thus suppress the synthesis of catecholamines, while promoting the synthesis of false neurotransmitters (ea. octopamine, and phenylethanolamine), as is observed in hepatic insufficiency (52). Increased brain tryptophan would result in enhanced synthesis of serotonin (53). Although plasma tryptophan was not increased, elevations in brain tryptophan have been observed in hepatic syndromes (with no increase in plasma tryptophan) (48,49). Since serotonin containing neurons are associated with suppressed behavior, while neurons containing catacholamines are associated with arousal, the observed changes in BCAA/AAA ratios may be related to the behavioral changes such as anorexia. The ratio of tyrosine to phenylalanine (T/P) was also reduced from 1.28 and 1.33 in the ad libitum and pair fed controls respectively to 0.92 in the aflatoxin treatments. Anderson has demonstrated that a correlation exists between a suppression in the T/P ratio and a decrease in feed intake (54). The 28% decrease in the T/P ratio in our data would be predicted to induce the 20% depression in feed intake observed in the aflatoxin groups by applying Anderson's model.

Taurine, which was also elevated in response to aflatoxin, is a component of the bile salt, taurocholic acid. Conjugation with taurocholic acid represents a major route for the excretion of aflatoxin and its metabolites in the chicken (34) and many other species (55). Dietary aflatoxin has been shown to reduce the concentration of bile salts in the bile, to but increase the volume of bile present in the gall bladder (45).

Elevated plasma levels of ammonia, glutamine, and perhaps BUN suggest a decreased ability to excrete nitrogen and/or an increased catabolism of protein or other nitrogenous compounds. Although excretion of nitrogen as urea is a minor pathway in the chicken, the decreased plasma concentration of uric acid together with the elevated arginine, and ornithine, (urea cycle intermediates) and perhaps BUN, suggests an increased importance of urea production in the excretion of nitrogen during aflatoxicosis. This observation will be discussed in detail in Chapter V. Changes in plasma amino acid concentrations can have numerous physiological effects which will be discussed in more detail in Chapters V & VI. Some of the symptoms of aflatoxicosis may be directly attributable to these plasma abnormalities.

CHAPTER IV

EFFECT OF CHOLINE AND FOLATE SUPPLEMENTATION

Introduction

A major aspect of aflatoxicosis in livestock involves the nutritional status of the affected animal (47,56). Aflatoxin interacts with protein, lipid, and vitamin metabolism, and also increases most nutritional requirements (32,57). Concentrations of thiamine, riboflavin, vitamin B6, pantothenate, niacin, biotin and choline decrease in the plasma, bile and liver of chicks fed aflatoxin contaminated feed. Only the concentration of folate increases in plasma and bile (47). Dosages of aflatoxin too low to inhibit the growth of chicks influence lipid synthesis and transport (58). Low dosages of aflatoxin also diminish the concentration of bile secreted and pancreatic lipase activity is decreased in proportion to the dosage of aflatoxin. These two factors combine to produce steatorrhea, a result of impaired digestion and absorption of lipophilic substances including the lipid soluable vitamins (45,46). The reduction in bile salts, which inhibit the growth of Gram positive bacteria, along with the alteration in the secretion of digestive enzymes, may result in the development of an atypical intestinal microflora (59) as well as hinder nutrient digestion or absorption. This suggestion is consistent with the observation that the administration of antibiotics, especially vitamin-antibiotic combinations to poultry with aflatoxicosis will improve feed conversion, weight gain, and mortality (56,60). These factors, together with aflatoxin induced anorexia, could be expected to produce nutritional deficiencies that may be responsive to dietary therapy.

A variety of vitamin and nutrient supplements or deficiencies have been reported to interact with aflatoxicosis. Isocaloric diets formulated on a constant calorie/protein ratio and high in lipid, provide a mortality sparing effect independent of the degree of saturation, but if the lipid also contains a high level of unsaturated fatty acids, there is also a significant growth promoting effect (60). Dietary supplementation with vitamin B6 improves feed conversion of chicks with aflatoxicosis but increased pantothenate has no effect (unpublished results, Voigt, Wyatt, and Ayres). Supplemental choline combined with B12 provides a protective effect to quail with aflatoxicosis (61). Dietary deficiency of thiamine, or supplementation with folic acid combined with vitamin B6 and vitamin D have been reported to offer mediation to poultry consuming aflatoxin (56,,42). In addition, diets marginally deficient in choline, methionine, and folic acid enhance hepatocarcinogenosis and depress hepatic mixed-function oxidase activities in rats fed aflatoxin B1 (62), while folic acid added to swine rations containing mouldy corn has been reported to improve growth and feed conversion (63).

The object of the first two feeding trials was to evalulate the influence of dietary folate or dietary and intraperitoneal (IP) choline on the aflatoxin lesion. Choline was selected to be evaluated as a mediative agent for aflatoxicosis for the following reasons: (a) to determine if aflatoxicosis induces a nutritional deficiency of choline, since low levels of choline occur in plasma during aflatoxicosis (47), (b) to verify reports of positive effects from administration of various choline-containing dietary supplements

(61,62), and (c) to determine if the fatty liver syndrome induced by aflatoxicosis can be attributed to a deficiency of choline, since choline deficiency will also induce fatty liver (32). Choline was administrated via IP injections, as well as through the diet, in an attempt to minimize the effects of any aflatoxin induced reduction in intestinal absorption of choline, although the enterohepatic circulation of bile prevents the total bypass of any effects attributable to malabsorption.

Like choline, folate functions in the biological reactions involving the transfer of single carbon units and is also a lipotrophic factor. Similarities between the metabolic effects of folate deficiency and aflatoxicosis suggested exploring a dietary trial with folate, ie. folacin is required for hemopoises, hemostasis and immune responses, all of which are affected by aflatoxin (65,66). Folate is also the only vitamin which is excreted at higher concentrations in the bile during aflatoxicosis (47).

Effects of choline on performance

The effect of dietary and IP choline supplements on the performance of chicks is shown in Table IV. Choline supplied in the diet, but not when given via IP injections, resulted in a 16% increase in the weights of birds stressed by aflatoxin or by pair-feeding, but only provided an 8% increase in the ad libitum controls. Dietary choline also produced an 11% increase in feed intake in the ad libitum controls, but no significant increase in the feed intake of the aflatoxin group. Supplemental choline is clearly not mediating the

Table IV. Effect of dietary and IP choline supplements on the performance of chicks with aflatoxicosis.

	Supplemental choline		
	[a] 0	[b] IP	[b] Diet
	Weight Gain (g)		
Aflatoxin	521±23D	480±22D	603±44BC
Pair-fed	530±34D	499±22D	616±45BC
Ad libitum	638±60BC	587±36C	689±33A
	Feed Intake (g)		
Aflatoxin	823±71BCD	728±61CDE	834±98BC
Pair-fed	796±67CDE	710±23E	792±82CDE
Ad libitum	928±65AB	875±78BC	1029±83A
	Feed Conversion (Feed intake/weight gain)		
Aflatoxin	1.73±0.2A	1.66±0.2A	1.49±0.1AB
Pair-fed	1.63±0.1AB	1.55±0.1AB	1.38±0.1B
Ad libitum	1.56±0.1AB	1.60±0.1AB	1.90±0.2AB

Values (mean and standard deviations) for a parameter followed by the same letter are not significantly different at $P \leq 0.05$. Evaluation for the occurance of interaction in the major effects is provided in Appendix Tables 1a-3a.

[a] No supplemental choline.

[b] Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provides 106%).

anorexic effects of aflatoxin, however the analysis of the effects of choline on aflatoxin toxicity is made difficult by the control response to choline supplementation. The 106% of the requirement of choline suggested by the NRC which was provided in the basal diet was not sufficient to provide maximal growth. This suggests that the NRC requirements for choline should be revised.

Effect of choline on hepatic lipid, and on selected biochemical constituents in plasma

The data on hepatic lipid content in Table V indicates that dietary choline significantly decreased hepatic lipid in the aflatoxin group, but not in either control group, while IP choline had no effect. As feed intake was actually increased by dietary choline in the aflatoxin group, the decreased hepatic lipid suggests a moderation of the toxic effects of aflatoxin.

Table VI compares the effect of IP and dietary choline supplements on the % change in selected plasma constituents between the aflatoxin and the pair fed controls. In all cases IP choline reduced the effect of aflatoxin, while dietary choline had little or no effect.

Similarly, IP choline reduced the effect of aflatoxin (see Table VII.) on glutamine, lysine, threonine, citrulline, ornithine, and tryptophan, while dietary choline had little or no effect. Arginine, tyrosine, phenylalanine, and serine were not affected by either supplement, while the increase in taurine was reduced by injected choline and further reduced by dietary choline. The tendency for choline, particularly IP choline, to normalize the plasma levels

Table V. Effect of dietary and IP choline on hepatic lipid content.

	Hepatic lipid (% DWB)		
Treatment	No choline	[a] IP choline	[a] Dietary choline
Aflatoxin	31.4+ 4.2A	32.1+ 4.2A	27.5+ 3.6B
Pair-fed	12.9+ 2.0C	14.3+ 2.8C	12.5+ 1.1C
Ad libitum	14.0+ 2.1C	14.4+ 1.4C	14.9+ 1.1C

Values (mean± standard deviations) for a parameter followed by the same letter are not significantly different at P≤0.05. Evaluation for the occurance of interaction in the major effects is provided in Appendix Table 16a.

[a] Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provides 106%).

Table VI. Effect of dietary and IP choline on the percent change of selected plasma constituents between the chicks receiving aflatoxin and the pair-fed controls.

	Aflatoxicosis vs. pair-feeding (% change)		
	[a] No choline	[b] IP choline	[b] Dietary choline
Calcium	-31 *	-21 *	-36 *
Phosphorus	-18 *	-2	-8
Cholesterol	-57 *	-52 *	-66 *
Total protein	-57 *	-29 *	-57 *
Total transferin iron	-32 *	-8 *	-39 *
LDH	+73 *	+40 *	+71 *

[a] No supplemental choline

[b] ~~Administration to achieve~~ 175% of the NRC requirement (1977) of choline (basal ration provides 106%).

* Percent change ([plasma constituent] in chicks reeiving aflatoxin / [plasma constituent] in pair-fed control chicks X 100) is significant at P≤0.05. ie. The concentration of the given plasma constituent in chicks receiving aflatoxin is significantly different at P≤0.05 from the same plasma constituent in the pair-fed controls. Raw data and evaluation for the occurance of interaction in the major effects is provided in Appendix Tables 17 and 17a respectively.

Table VII. Effect of dietary and IP choline on the percent change of selected plasma amino acids between the chicks receiving aflatoxin and the pair-fed controls.

	Aflatoxicosis vs. pair-feeding (% change)		
	[a] 0	[b] IP	[b] DIETARY
Glutamine	+78 *	+42 *	+78 *
Lysine	-17	-4	-31 *
threonine	-31	-12	-12
citrulline	-14	+13	-40
ornithine	+210 *	+140 *	+391 *
arginine	+71 *	+85 *	+126 *
tyrosine	+63 *	+53 *	+30 *
phenylalanine	+167 *	+171 *	+202 *
tryptophan	+2	-18 *	+14
serine	-20 *	-24 *	-25 *
cystathionine	-20	-33 *	+14
taurine	+153 *	+107 *	-7

[a] No supplemental choline.

[b] Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provides 106%).

* Percent change ([plasma constituent] in the aflatoxin chicks/[same constituent] in pair-fed controls X 100) is significant at $P \leq 0.05$. ie. The concentration of the given plasma constituent in chicks receiving aflatoxin is significantly different from the pair-fed controls.

of amino acids and other plasma constituents, suggests that nutrient absorption was improved by choline, perhaps by stimulating bile salt formation. The choline induced decreased in taurine is in agreement with increased taurocholic acid production.

Effects of folate

Data on the effect of supplemental folate on the performance and hepatic lipid content of chicks with aflatoxicosis are shown in Tables VIII and IX. Folate showed no protective action against aflatoxin. Similarly, folate supplementation had no significant effect on the plasma concentrations of the clinical parameters and amino acids measured (App. 18 and 22). However, it is interesting to note that it did produce a significant increase in 10 different amino acids (Table X) in the pair-fed controls which did not occur in the aflatoxin birds. It is known that certain antibiotics, and hormones of similar structure to aflatoxin, adversely affect the activity of folate. Perhaps aflatoxin has a similar effect.

Table VIII. Effect of supplemental folate on the performance of chicks with aflatoxicosis.

	Supplemental folate (% of NRC diatary requirement)		
	224	344	644
Treatment		Weight gain (g)	
Aflatoxin	435±24EF	474±21E	412±12F
Pair-fed	517±19D	564±65C	552±45CD
Ad libitum	673±15A	583±13BC	617±22B
		Feed intake (g)	
Aflatoxin	881±73CD	834±32D	927±78C
Pair-fed	884±57CD	831±38D	920±59C
Ad libitum	1096±48B	1174±84AB	1202±63A
		Feed conversion (g)	
Aflatoxin	2.23±0.3B	1.91±0.2CD	2.48±0.3A
Pair-fed	1.84±0.2D	1.59±0.2E	1.79±0.1DE
Ad libitum	1.72±0.0DE	2.15±0.2B	2.07±0.1BC

Values (mean ± standard deviations) for a parameter followed by the same letter are not significantly different at $P \leq 0.05$. Evaluation for the occurance of interaction in the major effects is provided in Appendix Tables 4a-6a.

Table IX. Effect of supplemental folate on hepatic lipid.

	Hepatic lipid (% dry weight basis)		
	Supplemental folate (% NRC dietary requirement)		
Treatment	244	344	644
Aflatoxin	27.4±2.1A	28.9±2.8A	28.2±2.4A
Pair-fed	15.9±3.2BC	17.5±1.1B	15.3±1.2BC
Ad libitum	14.1±1.2C	14.2±1.8C	13.9±1.4C

Values (mean± standard deviations) for a parameter followed by the same letter are not significantly different at $P \leq 0.05$. Evaluation for the occurance of interaction in the major effects is provided in Appendix Table 16a.

Table X. Percent change of selected plasma amino acids in chicks receiving a 164% increase in dietary folate.

	Basal vs. supplemental folate (% change)	
	Aflatoxin	Pair-fed
Asparagine	+2	+48*
Threonine	-9	+20*
Serine	+10	+37*
Glutamine	+4	+49*
Proline	+6	+18*
Glycine	+3	+25*
Alanine	-6	+24*
Valine	-2	+16*
Methionine	-8	+30*
Histidine	+11	+18*

* Percent change ([plasma amino acid] in chicks receiving supplemental folate / [plasma amino acid] in chicks receiving the basal ration X 100) is significant at P≤0.05. ie The plasma concentration of the given amino acid in plasma of the chicks receiving supplemental folate is significantly different from the concentration of the same plasma amino acid in chicks receiving the basal ration. Raw data and evaluation for the occurance of interaction in the major effects is provided in Appendix Table 22 and 22a respectively.

CHAPTER V

EFFECT OF LYSINE AND THREONINE SUPPLEMENTATION

Introduction

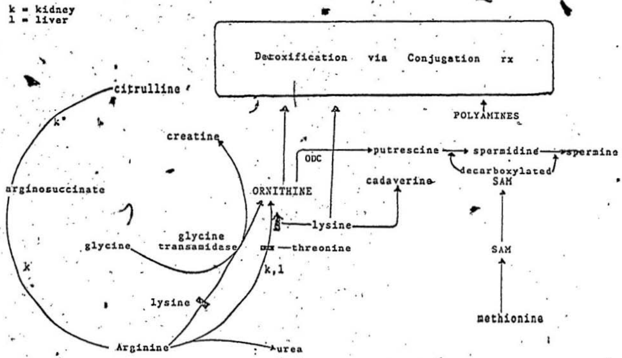
As a result of its toxic and carcinogenic effects, aflatoxin has caused severe economic losses to the animal industries. Poultry are susceptible to the acute toxicity of aflatoxin, but resistent to its carcinogenic effects. The level of in vivo binding of metabolites of aflatoxin B1 to DNA appears to reflect the susceptibility of a species to the carcinogenic action of the toxin, while the level of protein binding reflects susceptibility to the acutely toxic action of aflatoxin B1 (67). Acute toxicity in poultry is characterized by poor performance (decreased feed consumption, weight gain, and feed conversion 68,69), accumulation of fat in the liver (70), impaired protein synthesis (71), and increased susceptibility to infection (72).

Data presented in Chapter III has shown that abnormal plasma concentrations of certain amino acids or amino acid ratios are consistently induced by administration of 2.5 ug aflatoxin/g diet to Hubbard/Hubbard broiler chicks for 24 days. These changes included marked elevation of plasma phenylalanine, tyrosine, arginine, and taurine, while lysine and threonine concentrations were reduced. Deficiencies, excesses and imbalances of amino acids can lead to similar changes in plasma free amino acids (73) and, like aflatoxin, may cause a marked reduction in feed consumption and weight gain (74). A rise in plasma phenylalanine roughly equivalent to that induced by dietary aflatoxin has been reported to result in impaired protein synthesis and a decrease in antibody response (75,76). Accumulation of fat in the liver has also been reported to result from various plasma amino acid imbalances (77,78). If the effects of aflatoxin are related in part or in whole to these abnormal amino acid levels, the symptoms should be relieved if these concentrations could be normalized through appropriate dietary amino acid supplementation. Feeding trials 3 and 4 studied the effects of increasing increments of threonine or lysine on the performance and clinical biochemistry of chicks with aflatoxicosis.

Lysine and threonine were chosen because of the low concentrations of these amino acids in the plasma, and also because of the elevated levels of plasma arginine observed in the birds suffering from aflatoxicosis. Lysine competes with arginine for reabsorption in the renal tubules, and thus an excessive dietary intake of lysine can cause reduced arginine retention. In addition, lysine markedly increases renal arginase activity, thus stimulating the degradation of arginine to ornithine and urea (79,80). In contrast, threonine inhibits this reaction (80).

The resulting changes in ornithine production might also be expected to effect the symptoms of aflatoxicosis. Chickens do not reutilize ornithine for urea production as do ureatilic animals (Fig. 11). Instead ornithine is available to participate in detoxification through conjugation with a variety of xenobiotics, most notably benzoic acid. Ornithuric acid (dibenzoylornithine) is a major detoxification product in birds, snakes, and lizards, largely replacing the glycine conjugate hippuric acid which is excreted by mammals (81,82). Daily benzoic acid administration has been observed

Fig. 11. Role of ornithine in the formation of physiological nucleophiles


k = kidney
l = liver
Detoxification via Conjugation rx
POLYAMINES
citrulline
creatine
ODC
putrescine
spermidine
spermine
decarboxylated SAM
arginosuccinate
ORNITHINE
cadaverine
lysine
glycine
glycine transamidase
threonine
SAM
k
k,l
lysine
methionine
Arginine
urea

to increase urea nitrogen from 1% to 9% of the total nitrogen excreted by chickens. This rise in urea production was used to indicate the operation of the ornithine detoxification mechanism (82,83). Data presented in Chapter III showing an aflatoxin induced increase in plasma levels of ammonia, glutamine, possibly BUN, and the urea cycle intermediates, together with a decreased plasma concentration of uric acid, suggests a similar increased axcretion of nitrogen as urea in response to daily aflatoxin administration, and thus suggests a similar reliance on the ornithine detoxification mechanism.

In addition, ornithine, together with lysine and methionine, is a precursor of the polyamines (putrescine, spermidine, spermine, and cadaverine). Polyamines are also known to be excreted bound to such compounds as plant alkaloids, antibiotics of microbial nature, and various carboxylic acids (84). If these highly nucleophilic compounds facilitate the excretion of aflatoxin or its metabolites through the formation of conjugates, then changes in the plasma concentrations of these compounds could be expected to effect the performance of chicks with aflatoxicosis. Metabolites of aflatoxin are known to be excreted in bile and urine as conjugates of compounds such as taurine, and glucuronic acid (34,55). The metabolism of aflatoxin B1 by chicken liver microsomes has been reported to result almost exclusively in the formation of the 2,3-dihydroxy-2,3-dihydro-aflatoxin B1 (DHD-B1) metabolite of aflatoxin B1 (previously misidentified as the B2a or hemiacetyl derivative), which binds extensively to microsomal protein at neutral pH (85). Protein binding has also been observed *in vivo* with up to 63% of a given dose of aflatoxin reported to be concentrated in the muscle protein in broiler chicks (35), compared to 0.8% in piglets (33). It has been assumed that the dialdehydic phenolate ion of aflatoxin B1-dhd forms at physiological pH, and reacts with the primary amine groups of proteins to form a Schiff base (85). This same mechanism could apply to the formation of AFB1-dhd conjugates with the amine groups of free lysine, ornithine, arginine, and/or the polyamines. Thus the opposing effects of lysine and threonine on the production of ornithine and the polyamines provides a dual purpose for the choice of these two amino acids as dietary supplements to combine with dietary aflatoxin.

Contrasting effects of threonine and lysine on performance

Data on the effects of threonine and lysine supplementation on weight gains, feed conversion, and feed intake, are given in Tables XI and XII and XIII respectively. Lysine produced an increase in weight gain and feed conversion in the birds suffering from aflatoxicosis which was not observed in the pair-fed controls. Feed intake decreased in the controls, but remained unaffected in the aflatoxin treatments. In contrast, threonine failed to produce the beneficial effect on the body weight, feed conversion, and feed intake in the aflatoxin group, which is observed in the controls.

Lysine/arginine ratio

In Chapter III, plasma arginine concentration was shown to increase dramatically in response to aflatoxin, while plasma lysine

Table XI. Effect of graded levels of lysine or threonine on the final weight of chicks with aflatoxicosis.

Dietary supplement (% NRC requirement)		Final Weight (g) Aflatoxin	Pair-fed	Ad libitum
	102	367±32F	461±33C	545±47B
Lysine	122	411±25DE	446±13CD	586±22A
	146	402±20EF	448±11C	546±21B
	122	504±24EF	527±55DEF	620±39BC
Threonine	150	486±41F	547±17DE	628±24B
	175	519±36EF	573±59CD	729±40A

Values (means ± standard deviations) for a parameter followed by the same letter are not significantly different at $P \leq 0.05$. Evaluation for the occcurance of interaction in the major effects is provided in Appendix Tables 7a and 10a.

Table XII. Effect of graded levels of lysine or threonine on feed conversion of chicks with aflatoxicosis.

Dietary Supplement (% NRC requirement)		Feed Conversion (feed intake/weight gain)		
		Aflatoxin	Pair Fed	Ad Libitum
	102	2.55±0.11A	1.94±0.17E	2.07±0.14CDE
Lysine	122	2.23±0.08BC	2.03±0.06DE	2.16±0.30BCD
	146	2.26±0.21B	1.99±0.08DE	1.93±0.11E
	122	1.84±0.13ABC	1.65±0.07C-F	1.72±0.15B-E
Threonine	150	1.90±0.14A	1.61±0.06DEF	1.73±0.04BCD
	175	1.79±0.13ABC	1.50±0.08F	1.57±0.08EF

Values (means ± standard deviations) for a parameter followed by the same letter are not significantly different at $P \leq 0.05$. Evaluation for the occurance of interaction in the major effects is provided in Appendix Tables 9a and 12a.

Table XIII. Effect of graded levels of lysine or threonine on feed intake of chicks with aflatoxicosis.

Dietary supplement (% NRC requirement)		Feed intake (g)	
		Aflatoxin	Ad libitum
	102	839±61D	1050±22B
Lysine	122	830±52D	1180±69A
	146	821±56D	981±37C
	122	864±91B	1004±67A
Threonine	150	853±69B	1029±43A
	175	863±113B	1084±79A

Values (means ± standard deviations) for a parameter followed by the same letter are not significantly different at $P \leq 0.05$. Evaluation for the occurance of interaction in the major effects is provided in Appendix Tables 8a and 11a.

concentration decreased slightly. This aflatoxin induced decrease in plasma lysine/arginine ratio may be related to the negative effect of increased dietary threonine and the positive effect of increased dietary lysine on the performance of chicks receiving aflatoxin (Fig. 12 and 13). Fig. 13 shows that the 43% increase in dietary lysine increased the low plasma lysine/arginine ratio (0.48) in the birds suffering from aflatoxicosis to a more normal ratio (1.2). However, in the pair-fed and ad libitum controls, the ratios were increased to abnormally high ratios of 1.5, and 2.2, respectively, in response to increased dietary lysine. In contrast the already low lysine/arginine ratio (0.73) of the aflatoxin chicks was further reduced to 0.48 by the 40% increase in dietary threonine (Fig.12), while the control ratios were also decreased but remained within the normal range.

As previously mentioned, excess dietary lysine increases the degradation of arginine to ornithine and urea, while also decreasing arginine retention by competing for reabsorption by the renal tubules. This results in an increase in plasma lysine/arginine ratio and in dietary arginine requirement with a consequent decrease in performance which may be relieved by increased dietary arginine (79,80,86,87). Arginine requirement, and thus tolerance of excessive dietary lysine or arginine varies considerably from one strain of chicks to the next and even between families within strains (79,88). This variation appears to result from genetic differences in lysine metabolism (89). The selection of chicks with high arginine (H.A.), or low arginine (L.A.) requirement

Fig. 12. Effect of graded levels of threonine on plasma lysine/arginine ratio of chicks with aflatoxicosis.

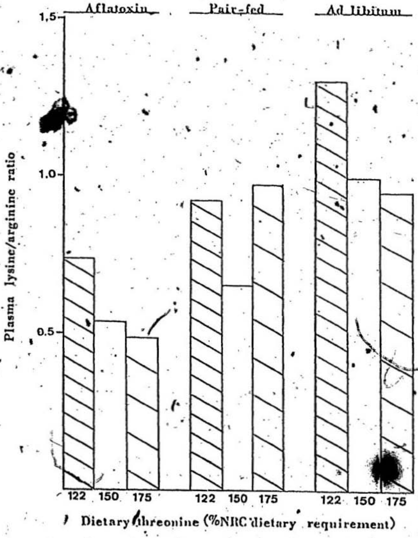
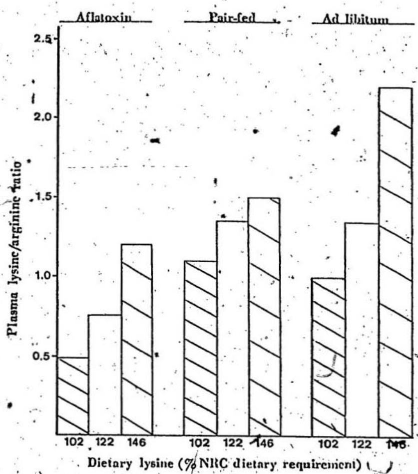

Aflatoxin
Pair-fed
Ad libitum
1.5
1.0
0.5
Plasma lysine/arginine ratio
122 150 175
122 150 175
122 150 175
Dietary threonine (%NRC dietary requirement)

Fig. 13. Effect of graded levels of lysine on plasma lysine/arginine ratio of chicks with aflatoxicosis.



Aflatoxin
Pair-fed
Ad libitum
2.5
2.0
1.5
1.0
0.5
Plasma lysine/arginine ratio
102
122
146
102
122
146
102
122
146
Dietary lysine (% NRC dietary requirement)

has produced two strains of chicks; Nesheim H.A and Nesheim L.A. (88). Regardless of the type of diet fed, plasma lysine levels in the H.A. strain were nearly double those of the L.A. strain, while plasma arginine levels showed little variation.

Susceptibility to aflatoxicosis also varies considerably from one strain of chicks to the next or even between individuals within a strain (2). In light of the effects of aflatoxin on the plasma lysine/arginine ratio in the Hubbard/Hubbard strain, and the improved performance seen after normalization of this ratio, it would be interesting to compare the susceptibility of the H.A. and L.A. strains to aflatoxicosis. The H.A. strain, already having a high lysine/arginine ratio, could be expected to be less harmed by the decrease in the ratio produced by aflatoxin, than would the L.A. strain, which already have a low plasma lysine/ arginine ratio. If these predictions are substantiated, this information could be of value in the selection of aflatoxin resistent chickens.

Contrasting effects of threonine and lysine on selected amino acids

Also of interest are the effects of lysine and threonine supplements on the plasma concentrations of ornithine and methionine (Table XIV). Predictably, the 43% increase in dietary threonine (inhibits arginase) caused a significant decrease in plasma ornithine concentration in both aflatoxin and pair-fed control groups. In contrast, the 43% increase in dietary lysine (stimulates arginase) caused an accumulation of ornithine in the pair-fed control birds, but in the aflatoxin group a decrease in ornithine concentration was

Table XIV. Effect of lysine and threonine supplements on plasma levels of selected amino acids in chicks with aflatoxicosis.

Amino Acid (nmole/ml of plasma)	Dietary Supplement (% NRC requirement)	Plasma amino acids (nmol/ml) Aflatoxin	Pair-fed	Ad libitum
Arginine	102 lysine	505±31A	399±111B	390±21BC
	122 lysine	468±24A	357±50BCD	386±51BC
	146 lysine	391±25BC	323±25D	344±25CD
Ornithine	102 lysine	74.0±8ABC	62.8±19BC	81.4±13AB
	122 lysine	93.6±21A	53.3±14C	71.6±12ABC
	146 lysine	69.1±5.7BC	78.8±12AB	75.5±16ABC
Methionine	102 lysine	75.1±6.7B	76.3±7.3B	80.3±4.8AB
	122 lysine	62.9±10C	86.6±5.2A	80.4±1.4AB
	146 lysine	59.1±6.5C	78.5±4.2AB	81.4±10AB
Arginine	122 threonine	553±46A	439±67BC	351±68DE
	150 threonine	532±53A	348±16DE	422±26C
	175 threonine	502±24AB	348±62E	414±45CD
Ornithine	122 threonine	133±5.3A	100±21B	56±20DE
	150 threonine	95±12B	38±7.7E	70±14CD
	175 threonine	86±7.3BC	52±22DE	76±4.6C
Methionine	122 threonine	54±5.3CD	73±9.8A	60±9.8BC
	150 threonine	52±8.6CD	54±5.0CD	68±6.2AB
	175 threonine	46±2.0D	61±8.7BC	61±4.8BC

Values (means ± standard deviations) for a parameter followed by the same letter are not significantly different at P≤0.05. Evaluation for the occurance of interaction in the major effects is provided in Appendix Tables 23a and 24a.

produced despite the large decrease in its precursor arginine which was observed in both the aflatoxin and control groups. This suggests that ornithine is being utilized more rapidly in the aflatoxin group. Methionine concentration was also decreased in response to increased lysine in the aflatoxin group but no change in methionine levels occurred in the control groups. Two alternate pathways for the utilization of ornithine and methionine include creatine synthesis and polyamine synthesis. Because lysine is known to inhibit hepatic glycine transamidase while increasing arginase activity (79,80), stimulation of creatinine synthesis in the presence of high concentrations of lysine is unlikely (Fig.11, p. 54). The polyamines (putrescine, spermidine, spermine, and cadaverine) are required for many important biochemical processes including protein synthesis (90). Thus the beneficial effects of lysine on the performance of chicks with aflatoxicosis may be due, at least in part, to its role in the stimulation of ornithine and/or polyamine synthesis. Similarly the inhibition of the production of these compounds by threonine may explain its negative effects on performance.

CHAPTER VI

EFFECT OF LYSINE/ARGININE SUPPLEMENTATION

Introduction

In the previous chapter, increased dietary lysine was shown to moderate the toxicity (as indicated by weight gain) but not the anorexic effects of aflatoxin, and it was suggested that the strain of chicken known as Nesheim H.A., having a comparatively high plasma lysine concentration, could be expected to be less susceptible to the effects of aflatoxin. As a follow up to this experiment, a fifth experiment was designed in which varying amounts of both lysine and arginine would be supplemented. In this way we hoped to relieve the lysine/arginine antagonism which was induced at the highest level of lysine supplementation in the previous experiment, and thus study the effects of increased plasma levels of the proposed detoxicants; lysine, arginine, ornithine, and the polyamines. Unexpectedly, the chicks used in this follow up experiment were found to differ quite markedly in their plasma levels of lysine, arginine, and lysine/arginine ratio (Table XV). The chicks from the lysine/arginine feeding trial appear to resemble quite closely the HA strain developed by Nesheim, which are characterized by high plasma lysine concentration and lysine/arginine ratio and hence a high dietary arginine (H.A.) requirement. In contrast the chicks in the former lysine experiment more closely resemble the LA strain developed by Nesheim, which are characterized by low plasma lysine concentration and lysine/arginine ratio, and hence a low dietary arginine (L.A.) requirement. The plasma lysine/arginine ratio of 0.99 reported by Nesheim (91) for the L.A. strain is remarkably

Table XV. Innate differences in the plasma concentrations of lysine, arginine, and lysine/arginine ratio in chicks from feeding trials 4 and 5.

	lysine experiment			lysine/arginine experiment		
	[a] Ad libitum Control	[c] Aflatoxin	[b] Average Control	[a] Ad libitum Control	[c] Aflatoxin	[d] Average Control
lysine	401±74	242±11	415	600±145	425±58	633
arginine	390±21	505±31	395	316±37	501±96	340
lys/arg	1.03	0.48	1.05	1.90	0.85	1.86

[a] Chicks recieving feed *Ad libitum* with no supplementation.
[b] Average of all non-aflatoxin groups recieving no lysine supplementation (40 chicks in total).
[c] Chicks recieving aflatoxin (2.5ug/g diet) with no aditional supplementation.
[d] Average of all non-aflatoxin groups (180 chicks in total).

By definition, the characteristics of the *ad libitum* controls recieving the basal ration (no supplementation) represent the innate characteristics of the entire group of chicks in each feeding trial. Thus the contrasting lysine/arginine ratio observed in the *ad libitum* controls from the two separate feeding trials indicates an innate difference in the two groups of chicks. This difference is also apparent when the aflatoxin groups from both feeding trials are compared, although both ratios have been reduced by aflatoxin.

In order to confirm that the twenty *ad libitum* control chicks are representative of the larger group of chicks in each feeding trial, the average control values, derived from a larger proportion of the total number of chicks, are also shown. These values correspond very closely with those of the *ad libitum* controls.

similar to the ratio of 1.03 observed in the ad libitum control chicks in the lysine feeding trial, while the ratio of 1.90 in the ad libitum control chicks from the lysine/arginine experiment more closely resemble the ratio of 3.06 reported by Nesheim for the H.A. strain.

Although unselected commercial broilers show great variation in arginine requirement, ranging from individuals which could be described as having low arginine requirement (L.A.) to those having high arginine requirement (H.A.), it is at first difficult to explain how two separate groups of chicks could be obtained from the same supplier, which differed so widely in this regard and yet showed relative uniformity within each group. However on closer examination, the finding that chicks of the H.A. strain hatch several hours sooner than chicks from the L.A. strain when eggs are treated the same during incubation; (92) provides a plausible explaination: it can easily be immagined that early and late hatching chicks, shipped immediately after hatched, would appear in separate shipments.

Relationship between plasma lysine/arginine ratio and performance

From this data, and the data presented in the previous chapter showing that elevated plasma lysine concentration reduced the toxic effects of aflatoxin, one might expect that the chicks used in the lysine/arginine experiment would be resistant to the toxic effects of aflatoxin. The feed intake and weight gain of the aflatoxin and control chicks are compared in Tables XVI. and XVII. respectively. As predicted, the chicks in the lysine/arginine experiment showed an anorexic effect in response to aflatoxin, but no toxic effect; weight

Table XVI. Effect of graded levels of lysine and arginine on feed intake of chicks receiving aflatoxin.

	Dietary Supplementation (% NRC requirement)			
	94 arg 102 lys	122 arg 102 lys	122 arg 122 lys	122 arg 146 lys
Treatment	Feed intake (g)			
Aflatoxin (2.5ug/g diet)	980±22B	898±56CD	930±104BCD	892±48CD
Ad libitum controls	1115±41A	1107±60A	1084±105A	1080±43A

Values (mean ± standard deviations) followed by the same letter are not significantly different at $P<0.05$. Evaluation for the occurance of interaction in thr major effects is provided in Appendix Table 13a.

Table XVII. Effect of graded levels of lysine and arginine on weight gain of chicks receieving aflatoxin.

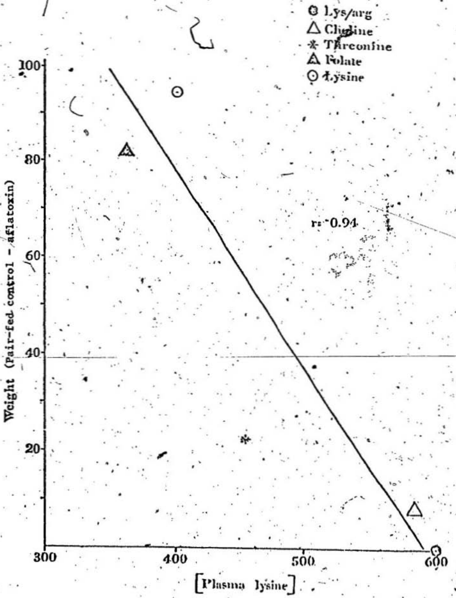
	Dietary Supplementation (% NRC requirement)			
	94 ARG 102 LYS	122 ARG 102 LYS	122 ARG 122 LYS	122 ARG 146 LYS
Treatment	Weight gain (g)			
Aflatoxin (2.5ug/g diet)	481±22DE	446±25EF	498±68CD	465±17DEF
Pair-fed controls	480±11DE	430±18F	482±21DE	439±22EF
Ad libitum controls	532±64BC	574±44AB	554±41AB	586±43A

Values (mean± standard deviations) followed by the same letter are not significantly different at P≤0.05. Evaluation for the occurance of interaction in the major effects is provided in Appendix Tables 14a.

gain is actually slightly higher in the aflatoxin group than in the pair fed controls!

Both plasma lysine concentration and the effect of aflatoxin on weight gain, varied considerably from one experiment to the next. Fig. 14 shows the relationship between these two factors. The difference in the final weight of the aflatoxin and pair fed controls receiving the basal ration, is plotted against the plasma lysine concentration in the *ad libitum* controls receiving the basal ration in each separate experiment. The correlation coefficient of -0.94 shows that there is a strong negative correlation between aflatoxin toxicity (as indicated by decreased weight gain over and above the decrease attributable to the anorexic effects of aflatoxin) and plasma lysine concentration, indicating that the Nesheim HA and genetically related chickens should be less susceptible to aflatoxin then the LA and related chickens.

Fig. 14. Relationship between plasma lysine concentration and aflatoxin toxicity.



Lys/arg
Choline
Threonine
Folate
Lysine
r= -0.94
Weight (Pair-fed control - aflatoxin)
100
80
60
40
20
300
400
500
600
[Plasma lysine]

CHAPTER VII

SUMMARY AND CONCLUSIONS

Aflatoxin has been shown to produce a decrease in the plasma levels of a wide range of nutrients, in a pattern indicative of a malabsorption syndrome. Previous workers have shown that dietary aflatoxin reduces bile concentration and reduces pancreatic lypase activity. More research is necessary to show a causal relationship between these two findings. Supplementary choline, particularly when administrated through IP injections, significantly reduced the effects of aflatoxin on the majority of plasma constituents, and thus appears to relieve the aflatoxin induced malabsorption. Dietary choline also significantly reduced hepatic lipid and weight gain in the aflatoxin birds.

Although aflatoxin produced an overall reduction in plasma nutrient concentrations, specific plasma constituents were consistently increased in response to aflatoxin, most notably taurine, phenylalanine, and to a lesser extent tyrosine, together with several other urea cycle intermediates. Specific amino acid imbalances, particularly the decrease in tyr/phe and BCAA/AAA may contribute to suppressed feed intake.

Previous researchers have observed daily benzoic acid administration to increase urea nitrogen from 1% to 9% of the total nitrogen excreted by chickens. This rise in urea production was used to indicate the operation of the ornithine detoxification mechanism. The aflatoxin induced increase in plasma levels of BUN, ammonia, glutamine and the urea cycle intermediates, together with the decreased plasma concentration of uric acid, suggests a similar increased excretion of nitrogen as urea in response to daily aflatoxin administration, and thus suggests a similar reliance on the ornithine detoxification mechanism.

The contrasting effects of lysine and threonine on the performance of chicks with aflatoxicosis may be related to their opposing effects on arginase, a key enzyme in the ornithine detoxification system. Regardless of the mechanism of the benificial effects of lysine, genetic variations in plasma lysine concentration may be of value in the selection of aflatoxin resistent chickens.

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APPENDIX A

RAW DATA TABLES 1--25

App. 1. Effect of dietary or interperitoneal (IP) administration of choline on the weight gain of chicks with aflatoxicosis.

	Weight Gain (g/chick)[a]								
	Aflatoxin (2.5 µg/g of diet)			Restricted Feeding[b]			Control		
Day	0[c]	IP[d]	Dietary[d]	0	IP	Dietary	0	IP	Dietary
1	40±1.6A	39±0.5A	42±1.0A	41±1.7A	40±1.5A	41±1.7A	41±20A	41±1.7A	39±1.0A
2	48±5.0A	46±4.7A	47±7.2A	44±1.0A	43±3.9A	45±5.4A	42±4.7A	42±1.3A	48±3.4A
3	62±8.6A	59±4.0A	62±7.8A	63±9.1A	60±3.4A	66±7.7A	60±3.2A	53±4.3A	65±4.4A
4	72±9.8A	71±5.0A	70±15A	75±5.7A	72±1.0A	80±7.3A	73±3.0A	68±6.8A	79±3.8A
5	87±8.3A	85±4.7A	90±9.5A	87±3.5A	86±4.9A	94±9.0A	86±3.4A	86±3.8A	96±4.9A
6	101±5.5AB	101±5.2AB	108±11AB	103±4.3AB	103±2.6AB	110±7.6AB	105±7.7AB	99±2.2B	112±6.6A
7	119±10A	115±6.1A	117±23A	117±4.9A	115±3.9A	122±7.3A	116±4.4A	112±5.3A	125±15A
8	141±4.8BCD	132±2.4DE	147±11AB	133±4.1CDE	130±2.9E	144±7.9ABC	133±5.4CDE	130±7.8E	154±12A
9	157±13ABC	149±3.2BC	161±20ABC	151±5.4BC	144±3.9BC	162±11AB	152±5.9BC	143±13C	173±14A
10	166±16ABC	157±9.6C	182±14A	157±16C	155±4.1C	178±16AB	162±8.4BC	157±9.4C	180±15AB
11	185±20BCD	165±7.8D	197±12AB	181±4.4B-D	175±4.8CD	194±17.7ABC	189±12BCD	179±13B-D	207±18A
12	211±5.3AB	194±19B	216±13AB	206±3.9AB	202±5.6AB	218±18.8AB	210±6.9AB	197±20B	227±24A
13	224±18A	215±13A	242±20A	229±6.7A	219±6.4A	244±22A	217±36A	213±28A	254±27A
14	246±17DE	241±10E	273±14B	258±8.5B-E	248±6.8CDE	269±24.7BC	267±18BCD	254±17B-E	297±9.4A
15	264±31B	269±14B	292±28AB	282±1.6AB	282±9.2AB	289±24.9AB	308±14A	291±16AB	312±21A
16	290±18CD	287±15D	327±20B	304±5.7BD	299±10.6BCD	316±31.5BC	325±16B	312±23B-D	358±26A
17	321±18BC	311±16BC	336±52AC	286±77C	326±11BC	344±33.8ABC	368±26AB	341±25ABC	393±29A
18	359±27CD	340±17D	380±10BC	367±8.1CD	363±5.5CD	382±38.5BC	407±30BC	391±22B	441±29A
19	379±18D	362±9.5D	386±51CD	393±7.6CD	375±10.8D	407±49.6BD	444±34AB	429±29ABC	472±39A
20	439±37CD	393±14E	443±8.1B-D	424±13DE	408±3.9DE	464±41BC	481±41AB	469±30BC	513±27A
21	427±22EF	396±9.1F	471±10CD	461±6.1DE	423±11F	506±58.7BC	515±39B	492±29BCD	556±24A
22	451±26D	408±13E	494±12BC	478±5.0CD	452±24D	520±54B	588±30A	507±28BC	600±32A
23	493±24E	467±17E	538±9.1CD	502±21DE	493±8.8E	581±59BC	602±47B	561±35BC	667±39A
24	521±23D	480±22D	603±44BC	530±34D	499±22D	616±45BC	638±60B	587±36C	689±33A

[a] Values (means ± standard deviations) for weight gain followed by the same letter are not significantly different at $P \le 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] No supplemental choline.

[d] Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provides 106%).

App. 1a. Major and interactive effects produced by dietary or intraperitoneal administration of choline on weight gain of chicks with aflatoxicosis.

Day	Weight gain (g/chick)[a]						ANOVA (F[Pr >F])[f]		
	Effect of feeding			Effect of administration					
	Aflatoxin[b]	Restricted[c]	Ad libitum	0[d]	IP[e]	Dietary[e]	Feed	Admin	Feed x Admin
1	-A	-A	-A	-A	-A	-A	0.01(0.99)	0.54(0.59)	1.89(0.14)
2	-A	-A	-A	-A	-A	-A	1.71(0.20)	1.53(0.24)	0.67(0.62)
3	-A	-A	-A	-A	-A	-A	1.08(0.36)	3.46(0.046)	0.66(0.63)
4	-A	-A	-A	-A	-A	-A	1.09(0.35)	2.17(0.13)	0.85(0.50)
5	-A	-A	-A	-A	0A	-A	0.40(0.67)	5.74(0.0084)	0.44(0.78)
6	-A	-A	-A	-A	-A	-A	0.55(0.58)	6.41(0.005)	0.41(0.80)
7	-A	-A	-A	-A	-A	-A	0.03(0.97)	1.58(0.22)	0.42(0.80)
8	-A	-A	-A	136A	131A	148A	1.10(0.35)	18.94(0.0001)	1.31(0.29)
9	-A	-A	-A	153B	146B	165A	0.38(0.69)	9.32(0.0008)	0.87(0.50)
10	-A	-A	-A	161B	156B	180A	0.52(0.60)	11.7(0.0002)	0.11(0.98)
11	-A	-A	-A	183B	173B	199A	1.27(0.30)	11.7(0.0002)	0.52(0.72)
12	-A	-A	-A	209AB	197B	220A	0.25(0.78)	7.08(0.0034)	0.41(0.80)
13	-A	-A	-A	223B	215B	246A	0.08(0.92)	5.56(0.0095)	0.28(0.89)
14	253B	258B	272A	257B	248B	279A	5.24(0.012)	14.0(0.0001)	0.70(0.60)
15	275B	284B	304A	-A	-A	-A	6.74(0.0042)	2.49(0.10)	0.68(0.61)
16	301B	306B	332A	306B	299B	334A	821(0.0016)	10.2(0.0005)	0.71(0.59)
17	323B	319B	367A	325A	326A	358A	6.19(0.0061)	2.84(0.070)	0.97(0.44)
18	360B	370B	413A	378B	365B	401A	17.6(0.0001)	7.53(0.0025)	0.48(0.75)
19	376B	392B	448A	405AB	389B	422A	17.5(0.0001)	3.30(0.052)	0.13(0.97)
20	425B	432B	488A	448B	423C	473A	18.6(0.0001)	9.84(0.0006)	0.63(0.65)
21	431A	463B	521A	468B	437C	511A	31.1(0.0001)	20.7(0.0001)	0.12(0.98)
22	451C	483B	563A	505B	456B	538A	51.6(0.0001)	25.7(0.0001)	1.03(0.41)
23	499C	525B	610A	532B	507C	595A	36.7(0.0001)	22.7(0.0001)	0.52(0.72)
24	534B	548B	638A	563B	522C	636A	27.3(0.0001)	28.5(0.0001)	0.27(0.89)

[a]Means for the gain in weights within a major effect followed by the same letter are not significantly different at P<0.05.

[b]Dietary aflatoxin 2.5μg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]No supplemental choline.

[e]Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provided 106%).

[f]Admin = Major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 2. Effect of dietary or interperitoneal (IP) administration of choline on the feed intake of chicks with aflatoxicosis.

	Feed intake (g/chick)[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
Day	0[c]	IP[d]	Dietary[d]	0	IP	Dietary	0	IP	Dietary
1	0.0±0.0A	0.0±0.0A	0.0±0.0A	0.0±0.0A	0.0±0.0A	0.0±0.0A	0.0±0.0A	0.0±0.0A	0.0±0.0A
2	8.5±1.7A	7.5±2.9A	7.0±4.1A	7.5±1.3A	7.8±2.6A	9.3±3.0A	4.8±1.5A	7.5±3.9A	8.5±1.3A
3	21±2.4A-C	16±4.8BC	19±6.2BC	28±6.1A	18±4.3BC	22±4.1AB	13±4.7C	15±6.0BC	22±2.5AB
4	36±3.9A	30±4.9A	33±8.3A	43±6.5A	34±5.9A	42±13A	29±5.5A	30±7.7A	39±4.4A
5	54±4.7A	47±4.9A	55±19A	63±8.2A	56±8.2A	53±10A	46±6.1A	48±7.8A	59±5.2A
6	76±5.3A	67±5.6A	76±23A	81±8.4A	73±8.4A	72±8.4A	66±6.5A	67±8.9A	81±5.5A
7	100±7.1A	89±5.6A	101±25A	103±7.9A	93±8.6A	94±9.0A	89±6.0A	93±11A	110±6.6A
8	124±6.9AB	114±6.0B	128±25AB	126±9.1AB	115±9.2B	120±9.8AB	116±5.9B	120±12AB	140±7.7A
9	154±7.2A	141±7.0A	156±29A	153±11A	140±9.9A	147±10A	154±20A	150±14A	166±17A
10	183±7.8A	165±9.0A	184±31A	183±12A	167±11A	176±13A	183±21A	177±17A	198±19A
11	217±9.5AB	192±12B	215±32AB	212±14AB	193±17B	203±14AB	218±22AB	210±21AB	236±19A
12	248±8.5AB	222±12B	241±31AB	246±16AB	220±21B	233±15B	253±26AB	248±25AB	275±20A
13	288±13A-C	265±21BC	276±37ABC	278±16A-C	250±20C	259±16BC	295±28AB	290±31A-C	308±17A
14	320±14A-C	279±24C	311±41BC	314±15BC	285±23C	294±21BC	336±31AB	327±36AB	357±21A
15	360±14BC	315±27D	355±47BCD	347±15BCD	314±25D	330±25CD	384±33AB	372±40BC	418±22A
16	399±17BCD	378±42CD	398±52BCD	387±17BCD	350±28D	374±31CD	434±34AB	415±48BC	474±22A
17	442±22BCD	392±48D	440±57BCD	426±21BCD	387±30D	416±38CD	481±38AB	461±51BC	532±23A
18	492±26BCD	435±58DE	490±63BCD	469±25CDE	427±31E	464±40CDE	539±41AB	512±52BC	598±24A
19	545±32BC	473±52D	547±74BC	519±30CD	469±28D	516±51CD	560±46A	571±54BC	666±26A
20	601±39BC	528±58D	601±79BC	571±36CD	508±24D	574±60CD	666±50B	634±60BC	739±31A
21	652±46BC	573±59D	654±82BC	628±43DC	563±24D	631±63CD	727±57AB	688±71BC	802±35A
22	708±52CD	622±59DE	711±90C	679±51CDE	605±27E	678±69CDE	799±58B	749±70BC	904±71A
23	770±65BCD	675±60DE	767±95BCD	738±60CDE	654±29E	735±77CDE	863±64AB	809±74BC	955±80A
24	823±71BCD	726±61DE	834±98BC	796±67CDE	710±23E	792±82CDE	928±65AB	875±78BC	1029±83A

[a] Values (means ± standard deviations) for feed intake followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair-fed to the feed intake of the corresponding aflatoxin group.

[c] No supplemental choline.

[d] Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provides 106%).

App. 2a. Major and interactive effects produced by dietary or intraperitoneal (IP) administration of choline on feed intake of chicks with aflatoxicosis.

Day	Weight gain (g/chick)[a]						ANOVA (F [Pr > F])[f]		
	Effect of feeding			Effect of administration					
	Aflatoxin[b]	Restricted[c]	Ad libitum	0[d]	IP[e]	Dietary[e]	Feed	Admin	Feed x Admin
2	-A	-A	-A	-A	-A	-A	0.66(0.52)	0.75(0.48)	1.09(0.38)
3	-A	-A	-A	-A	-A	-A	4.59(0.019)	3.50(0.045)	2.91(0.040)
4	-A	-A	-A	-A	-A	-A	3.48(0.045)	2.52(0.099)	1.07(0.39)
5	-A	-A	-A	-A	-A	-A	1.63(0.21)	1.11(0.34)	1.72(0.18)
6	-A	-A	-A	-A	-A	-A	0.40(0.67)	1.54(0.23)	1.56(0.22)
7	-A	-A	-A	-A	-A	-A	0.01(0.99)	2.38(0.11)	1.87(0.15)
8	-A	-A	-A	122AB	116B	129A	0.53(0.59)	3.65(0.04	1.77(0.16)
9	-A	-A	-A	A	A	A	1.31(0.29)	2.34(0.12)	0.37(0.83)
10	-A	-A	-A	183AB	170B	186A	1.44(0.25)	3.11(0.06)	0.47(0.76)
11	208AB	202B	221A	215A	198B	218A	3.16(0.059)	3.72(0.037)	0.63(0.64)
12	237B	233B	259A	249A	230B	249A	5.62(0.0091)	3.56(0.043)	0.82(0.52)
13	276B	262B	298A	-A	-A	-A	6.94(0.0037)	1.94(0.16)	0.54(0.71)
14	303B	298B	340A	323A	297B	321A	9.05(0.0010)	3.64(0.041)	0.84(0.51)
15	343B	330B	391A	364A	334B	367A	14.4(0.0001)	4.81(0.016)	0.98(0.43)
16	392B	370B	441AB	407AB	381B	415A	13.3(0.0001)	3.21(0.056)	0.77(0.56)
17	425B	410B	491A	450A	413B	463A	14.1(0.0001)	4.91(0.015)	0.71(0.56)
18	472B	453B	550A	500A	458B	517A	17.6(0.0001)	6.23(0.0059)	0.81(0.53)
19	521B	501B	612A	554A	504B	577A	20.1(0.0001)	7.91(0.0020)	0.81(0.53)
20	577B	551B	679A	613A	556B	638A	21.2(0.0001)	7.95(0.0019)	0.66(0.63)
21	626B	607B	739A	669A	608B	696A	19.3(0.0001)	7.86(0.0020)	0.58(0.68)
22	680B	654B	817A	728A	659B	764A	23.4(0.0001)	8.75(0.001)	0.98(0.43)
23	737B	709B	876A	790A	713B	819A	20.2(0.0001)	7.60(0.0024)	0.64(0.64)
24	797B	766B	944A	850A	771B	885A	20.6(0.0001)	7.80(0.0021)	0.67(0.62)

[a]Means for feed intakes within a major effect followed by the same letter are not significantly different at P≤.05.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]No supplemental choline.

[e]Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provided 106%).

[f]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 3. Effect of dietary or interperitoneal (IP) administration of choline on the feed conversion of chicks with aflatoxicosis.

	Feed conversion ratio[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
Day	0[c]	IP[d]	Dietary[d]	0	IP	Dietary	0	IP	Dietary
2	1.39±1.1A	1.54±0.71A	0.81±2.0A	3.05±2.0A	2.81±1.9A	1.17±8.2A	0.84±3.7A	3.21±7.0A	1.11±0.43A
3	1.00±0.29A	0.82±0.16A	0.97±0.16A	1.41±0.76A	0.86±0.15A	0.96±0.37A	0.75±0.33A	1.28±0.22A	0.89±0.20A
4	1.16±0.26A	0.97±0.19A	1.43±0.73A	1.26±0.23A	1.07±0.21A	1.07±0.24A	0.90±0.20A	1.14±0.27A	0.96±0.05A
5	1.17±0.11A	1.04±0.10A	1.13±0.18A	1.36±0.21A	1.23±0.19A	1.13±0.33A	1.04±0.13A	1.09±0.30A	1.04±0.11A
6	1.26±0.10A	1.09±0.13A	1.13±0.17A	1.30±0.17A	1.16±0.14A	1.05±0.25A	1.05±0.22A	1.15±0.17A	1.11±0.06A
7	1.27±0.14A	1.19±0.11A	1.38±0.26A	1.36±0.17A	1.24±0.10A	1.16±0.17A	1.20±0.11A	1.32±0.11A	1.30±0.12A
8	1.23±0.04A	1.23±0.06A	1.20±0.13A	1.36±0.14A	1.27±0.11A	1.17±0.13A	1.26±0.08A	1.27±0.18A	1.23±0.09A
9	1.32±0.12A	1.28±0.06A	1.32±0.14A	1.39±0.16A	1.35±0.12A	1.22±0.13A	1.38±0.09A	1.47±0.11A	1.26±0.18A
10	1.46±0.17AB	1.40±0.05AB	1.30±0.11B	1.59±0.19A	1.45±0.09AB	1.29±0.14B	1.52±0.09AB	1.53±0.16AB	1.43±0.26AB
11	1.52±0.23A	1.55±0.18A	1.38±0.10A	1.51±0.07A	1.42±0.10A	1.33±0.16A	1.52±0.14A	1.53±0.16A	1.42±0.16A
12	1.45±0.07AB	1.45±0.12AB	1.38±0.10AB	1.48±0.07AB	1.35±0.10AB	1.32±0.14B	1.49±0.11AB	1.62±0.22A	1.48±0.12AB
13	1.57±0.17AC	1.52±0.13ABC	1.37±0.07BC	1.47±0.05ABC	1.39±0.08BC	1.29±0.14C	1.76±0.49A	1.70±0.11AB	1.45±0.18ABC
14	1.56±0.15A	1.38±0.07BCD	1.35±0.11CD	1.45±0.06AD	1.36±0.07CD	1.30±0.14D	1.49±0.11ABC	1.54±0.11AB	1.39±0.06BCD
15	1.64±0.28A	1.37±0.05BD	1.42±0.13A-C	1.44±0.07A-C	1.29±0.06C	1.34±0.13BC	1.44±0.08ABC	1.44±0.10ABC	1.54±0.16AB
16	1.60±0.17A	1.53±0.12AB	1.39±0.12AB	1.47±0.08AB	1.35±0.06B	1.37±0.15B	1.53±0.12AB	1.53±0.10AB	1.49±0.13AB
17	1.58±0.12A	1.44±0.19A	1.51±0.12A	1.46±0.94A	1.35±0.09A	1.38±0.15A	1.47±0.08A	1.53±0.10A	1.51±0.15A
18	1.55±0.14A	1.45±0.21A	1.44±0.17A	1.44±0.07A	1.32±0.09A	1.36±0.13A	1.47±0.07A	1.46±0.10A	1.49±0.12A
19	1.61±0.14A	1.47±0.18A	1.55±0.10A	1.47±0.09A	1.40±0.13A	1.42±0.12A	1.49±0.07A	1.47±0.08A	1.55±0.12A
20	1.52±0.13A	1.50±0.19A	1.50±0.19A	1.49±0.10A	1.38±0.06A	1.36±0.10A	1.52±0.07A	1.48±0.08A	1.57±0.14A
21	1.69±0.13A	1.61±0.19AB	1.52±0.19AB	1.49±0.10AB	1.47±0.06AB	1.36±0.10B	1.53±0.07AB	1.53±0.08AB	1.56±0.14AB
22	1.73±0.17A	1.68±0.16AB	1.57±0.18A-C	1.55±0.10ABC	1.47±0.07BC	1.42±0.11C	1.47±0.06BC	1.60±0.15A-C	1.61±0.13ABC
23	1.70±0.18A	1.58±0.11AB	1.54±0.18AB	1.60±0.11AB	1.44±0.12AB	1.36±0.11B	1.54±0.14AB	1.56±0.08AB	1.53±0.14AB
24	1.72±0.18A	1.66±0.18A	1.49±0.18AB	1.63±0.08AB	1.55±0.06AB	1.38±0.10B	1.56±0.07AB	1.60±0.08AB	1.59±0.23AB

[a] Values (means ± standard deviations) for feed conversion (feed intake/weight gain) followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] No supplemental choline.

[d] Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provides 106%).

App. 3a. Major and interactive effect produced by dietary or intraperitoneal (IP) administration of choline on the feed conversion ratio of chicks with aflatoxicosis.

Day	Feed Conversion ratios[a]						ANOVA (F[Pr > F])[f]		
	Effect of feeding			Effect of administration					
	Aflatoxin[b]	Restricted[c]	Ad libitum	0[d]	IP[e]	Dietary[e]	Feed	Admin	Feed x Admin
2	-A	-A	-A	-A	-A	-A	0.20(0.82)	1.48(0.23)	0.34(0.85)
3	-A	-A	-A	-A	-A	-A	0.69(0.51)	0.40(0.68)	2.21(0.095)
4	-A	-A	-A	-A	-A	-A	1.08(0.36)	0.25(0.78)	1.47(0.24)
5	-A	-A	-A	-A	-A	-A	1.70(0.20)	1.08(0.35)	1.10(0.38)
6	-A	-A	-A	-A	-A	-A	0.50(0.61)	1.25(0.30)	1.25(0.31)
7	-A	-A	-A	-A	-A	-A	0.09(0.91)	0.12(0.89)	1.64(0.19)
8	-A	-A	-A	-A	-A	-A	0.91(0.42)	2.12(0.14)	1.01(0.42)
9	-A	-A	-A	1.37A	1.37A	1.26A	0.88(0.43)	2.57(0.095)	1.19(0.34)
10	-A	-A	-A	1.52A	1.45AB	1.34B	1.40(0.26)	4.65(0.018)	0.56(0.70)
11	-A	-A	-A	1.52A	1.49AB	1.38B	0.66(0.52)	2. 7(0.068)	0.15(0.96)
12	1.43AB	1.39AB	1.53A	-A	-A	-A	3.41(0.048)	1.35(0.28)	0.79(0.54)
13	1.49AB	1.38B	1.64A	1.60A	1.54AB	1.37B	4.81(0.016)	4.33(0.023)	0.18(0.95)
14	1.43AB	1.37B	1.47A	1.50A	1.43A	1.34B	2.98(0.068)	7.16(0.0032)	1.28(0.30)
15	1.48A	1.36B	1.49A	-A	-A	-A	3.52(0.04)	2.40(0.11)	1.88(0.14)
16	1.40B	1.51A	1.52A	1.53A	1.47AB	1.42B	3.76(0.036)	2.91(0.072)	0.84(0.51)
17	-A	-A	-A	-A	-A	-A	0.10(0.90)	1.63(0.21)	1.34(0.28)
18	-A	-A	-A	-A	-A	-A	2.54(0.098)	1.08(0.35)	0.34(0.87)
19	1.56A	1.43B	1.50AB	-A	-A	-A	3.49(0.045)	1.59(0.22)	0.50(0.74)
20	1.5 A	1.41A	1.52A	-A	-A	-A	2.77(0.081)	0.60(0.55)	0.60(0.67)
21	1.61A	1.44B	1.54AB	-A	-A	-A	4.72(0.018)	1.47(0.25)	0.65(0.63)
22	1.66A	1.48B	1.56AB	-A	-A	-A	5.51(0.0098)	0.58(0.57)	1.77(0.16)
23	1.61A	1.47B	1.54AB	1.61A	1.53AB	1.48B	2.94(0.070)	2.73(0.084)	0.72(0.59)
24	-A	-A	-A	1.63A	1.60AB	1.49B	1.60(0.22)	3.64(0.040)	1.23(0.32)

[a]Means of feed conversion ratios (feed intake/weight gain) within a major effect followed by the same letter are not significantly different at P≤0.05.

[b]Dietary aflatoxin 2.5μg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]No supplemental choline.

[e]Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provided 106%).

[f]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 4. Effect of graded levels of dietary folic acid on the weight gain of chicks with aflatoxicosis.

	Weight gain (g/chick)[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
Day	244[c]	344[c]	644[c]	244	344	644	244	344	644
0	39±1.8A	37±1.5A	38±0.73A	37±1.5A	38±0.19A	37±0.79A	37±1.2A	37±1.3A	37±0.79A
1	49±2.2A	47±2.0A	49±1.2A	47±2.4A	47±0.69A	47±0.66A	48±1.6A	47±1.9A	48±2.0A
2	57±2.7A	55±3.2A	58±0.7A	57±3.3A	57±1.1A	58±1.5A	57±2.1A	56±2.9A	56±2.9A
3	68±2.2A	77±23A	68±1.0A	66±4.4A	69±2.8A	68±2.5A	67±3.8A	66±3.9A	66±4.0A
4	79±4.8A	76±3.0A	77±2.6A	78±6.0A	82±1.5A	81±1.4A	79±4.2A	78±4.8A	76±4.1A
5	93±2.4A	88±5.7A	89±10A	92±6.5A	95±0.67A	94±2.0A	93±4.5A	94±7.3A	92±5.6A
6	104±7.3AB	101±6.7B	101±3.8B	108±6.3AB	111±2.2A	109±2.6AB	110±5.8A	105±2.0AB	108±6.7AB
7	128±7.1A	119±7.3BC	119±2.8C	129±6.0A	127±2.7AC	128±5.3AB	128±1.5AC	123±2.5AC	129±6.9A
8	142±8.0A–C	136±8.1BC	133±3.6C	148±6.9A	147±5.3A	146±2.8A	145±3.5AB	140±6.3ABC	147±9.0A
9	156±8.8A–C	154±7.0BC	146±4.4C	162±2.6AB	162±7.5AB	166±4.0A	162±4.0AB	156±8.7ABC	166±11A
10	171±9.3CD	169±9.6CD	163±2.6D	180±3.9ABC	181±9.4ABC	186±6.3AB	183±4.6AB	174±10BD	189±12A
11	191±12C	189±9.8CD	178±4.9D	208±3.2AB	216±8.7A	211±9.3AB	209±5.0AB	200±11BC	215±11A
12	202±12CD	208±9.2C	192±3.2D	231±5.0AB	239±6.9A	236±10A	236±7.6A	221±6.7B	242±10A
13	217±12D	221±9.1D	203±3.6E	249±11BC	254±12ABC	250±9.8BC	261±11AB	244±7.8C	266±11A
14	233±13DE	238±9.9D	216±3.9E	268±18C	271±19BC	267±13C	287±16AB	270±8.0BC	295±13A
15	250±12C	257±13C	232±4.7D	301±9.7B	303±12B	308±17B	332±24A	297±7.3B	328±14A
16	259±6.3E	281±12D	251±4.6E	326±14C	330±13BC	342±19BC	372±24A	324±14C	347±25B
17	286±8.7CD	304±14C	271±5.7D	362±29B	358±19B	361±35B	408±27A	363±4.8B	393±15A
18	304±9.7DE	325±16D	288±5.2F	382±8.7CD	377±31C	400±31BC	440±25A	387±6.8C	416±22AB
19	328±12DE	351±17D	311±5.9E	416±7.6BC	404±28C	433±32B	483±24A	417±12BC	462±21A
20	341±16F	373±19E	328±6.9F	435±13CD	417±22D	474±18B	519±25A	448±14C	498±23A
21	369±17EF	395±22E	355±8.6F	447±16CD	433±19D	493±25B	561±26A	470±26BC	539±26A
22	391±16G	420±22F	374±9.5G	467±16E	45[illegible]±21E	497±13D	605±17A	521±11C	563±9.9B
23	402±35G	457±22F	411±27G	503±14DE	499±18E	534±52CD	659±14A	564±13C	607±12B
24	435±24EF	474±21E	412±12F	517±19D	564±65C	552±45CD	673±15A	583±13BC	617±22B

[a] Values (means ± standard deviations) for weight gain followed by the same letter are not significantly different at P ≤ 0.05.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of folic acid (basal ration provides 244%).

App. 4a. Major and interactive effects produced by graded levels of dietary folic acid acid on the weight gain of chicks with aflatoxicosis.

Day	Weight gain (g/chick)[a] Effect of feeding: Aflatoxin[b]	Restricted[c]	Ad libitum	Effect of administration[d]: 244	344	644	ANOVA(F[Pr >F])[e]: Feed	Admin	Feed x Admin
0	-A	-A	-A	-A	-A	-A	0.95(0.40)	0.53(0.59)	0.82(0.52)
1	-A	-A	-A	-A	-A	-A	1.66(0.21)	1.20(0.32)	0.36(0.83)
2	-A	-A	-A	-A	-A	-A	0.36(0.70)	0.46(0.64)	0.74(0.57)
3	-A	-A	-A	-A	-A	-A	0.96(0.39)	0.64(0.54)	0.47(0.76)
4	-A	-A	-A	-A	-A	-A	2.04(0.15)	0.01(0.99)	1.07(0.39)
5	-A	-A	-A	-A	-A	-A	1.79(0.19)	0.16(0.85)	0.65(0.65)
6	102B	109A	108A	-A	-A	-A	7.07(0.003)	0.36(0.70)	0.82(0.53)
7	122B	128A	126AB	128A	123B	125AB	4.25(0.023)	3.19(0.057)	1.39(0.26)
8	137B	147A	144A	-A	-A	-A	8.48(0.0014)	1.24(0.31)	0.95(0.45)
9	152B	164A	162A	-A	-A	-A	9.45(0.0008)	0.50(0.61)	2.07(0.11)
10	268B	282B	282B	-A	-A	-A	12.6(0.0001)	0.99(0.39)	2.02(0.12)
11	185B	212A	208A	-A	-A	-A	28.9(0.0001)	0.07(0.93)	2.99(0.036)
12	200B	235A	233A	-A	-A	-A	66.8(0.0001)	0.01(0.99)	6.03(0.001)
13	214B	251A	257A	-A	-A	-A	[illegible].4(0.0001)	0.23(0.80)	4.41(0.007)
14	229C	268B	284A	-A	-A	-A	55.4(0.0001)	0.22(0.81)	3.29(0.026)
15	246C	304B	319A	-A	-A	-A	94.9(0.0001)	1.66(0.33)	5.23(0.0030)
16	264C	332B	348A	-A	-A	-A	92.0(0.0001)	0.64(0.54)	6.54(0.0008)
17	287C	361B	388A	-A	-A	-A	80.5(0.0001)	1.08(0.35)	3.40(0.023)
18	306C	386B	414A	-A	-A	-A	97.3(0.0001)	1.26(0.30)	5.52(0.0022)
19	330C	418B	454A	409A	391B	402AB	128(0.0001)	2.67(0.088)	7.85(0.0002)
20	347C	442B	488A	431A	412B	433A	190(0.0001)	4.93(0.015)	14.3(0.0001)
21	373C	458B	523A	459A	433B	402A	150(0.0001)	6.93(0.0037)	12.6(0.0001)
22	395C	474B	563A	488A	466B	478AB	343(0.0001)	5.94(0.0073)	19.6(0.0001)
23	423C	512B	610A	441C	544B	624A	155(0.0001)	1.04(0.37)	9.90(0.0001)
24	441C	544B	624A	A	A	A	108(0.0001)	0.80(0.46)	7.26(0.0004)

[a]Means for the gain weights within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin = 2.5ug per g of diet.

[c]Pair-fed to the intake of the corresponding (1977) of folic acid (basal ration provided 244%).

[d]Percentage of the NRC dietary requirement (1977) of folic acid (basal ration provided 244%).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App . 5. Effect of graded levels of dietary folic acid on the feed intake of chicks with aflatoxicosis.

	Feed intake (g/chick)[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
Day	244[c]	344[c]	644[c]	244	344	644	244	344	644
1	10±0.67A	9.0±0.68AB	9.9±0.58A	8.1±0.69BC	7.5±0.72C	8.0±1.3BC	10±1.7A	10±0.55A	9.0±0.7ABC
2	21±0.70AB	20±2.0BC	21±1.2AB	17±1.0D	18±1.2CD	18±1.8CD	22±1.7A	21±1.6AB	20±2.1AB
3	37±1.1AB	35±3.6BC	37±2.3AB	30±1.7D	33±1.3CD	31±0.40D	37±0.99AB	39±2.8A	37±3.4AB
4	57±3.3AB	53±6.8BCD	57±5.1AB	46±2.1E	49±2.3CDE	47±0.66DE	55±2.7BC	62±7.3A	57±5.1AB
5	79±8.0BC	72±8.2BC	82±8.5B	68±7.2C	69±2.0C	72±4.8BC	78±6.6BC	94±12A	82±3.8B
6	103±9.0BCD	91±9.1CD	106±13ABC	91±8.8CD	88±2.1D	99±11BCD	100±8.5BCD	120±17A	110±5.9AB
7	131±12BC	118±9.5C	141±18AB	119±11C	115±2.6C	132±13BC	130±12BC	152±20A	148±5.2AB
8	166±8.0BCD	149±8.1CD	179±3.6AB	154±6.9CD	164±5.3D	170±16ABC	162±13BCD	189±23A	190±6.7A
9	206±19BCD	183±13DE	220±24ABC	202±21CDE	179±6.1E	211±21ABC	198±13CDE	231±26AB	234±9.8A
10	247±24BCD	220±11D	269±35ABC	236±32CD	216±6.0D	257±30ABC	240±13CD	284±32AB	287±13A
11	288±28BCD	257±13D	318±40AB	276±30CD	255±12D	308±33ABC	276±11CD	334±38A	337±15A
12	332±35BCD	296±14D	372±44AB	323±29CD	293±13D	363±35ABC	319±15CD	391±52A	394±20A
13	382±44BCD	339±20D	427±53AB	373±31BCD	335±20D	418±43ABC	365±22CD	449±68A	455±22A
14	432±54BC	384±25C	483±63AB	427±32BC	381±27C	473±52AB	424±22BC	520±77A	528±23A
15	484±57BC	432±36C	537±67AB	479±34BC	429±39C	526±55AB	481±27BC	582±83AB	585±26A
16	523±58BC	474±41C	575±71AB	517±33BC	472±44C	564±60AB	544±31BC	639±79A	635±28A
17	560±60BC	511±37C	611±72B	554±35BC	509±41C	600±61B	598±34B	697±82A	695±29A
18	595±60BC	548±38C	650±69B	590±34BCD	545±42C	639±58B	656±34B	753±86A	749±36A
19	638±61CD	588±38D	693±79BC	632±30CD	586±42D	685±60BC	720±35B	814±87A	812±36A
20	682±63CD	626±26D	735±78BC	676±31CD	624±30D	728±60BC	790±36B	878±87A	881±35A
21	724±65CD	674±24D	784±78C	722±36CD	671±29D	776±59C	863±37B	945±88A	952±39A
22	770±67CD	723±28D	830±79C	769±40CD	720±34D	882±59C	938±38B	1016±89A	1025±45A
23	820±70CD	771±26D	876±79C	819±46CD	769±33D	869±60C	1011±37B	1084±86AB	1125±56A
24	881±73CD	834±32D	927±78C	884±57CD	831±38D	920±59C	1096±48B	1174±84AB	1202±63A

[a] Values (means ± standard deviations) for feed intake followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of folic acid (basal ration provides 244%).

App. 5a. Major and interactive effects produced by graded levels of dietary folic acid on the feed intake of chicks with aflatoxicosis.

Day	Feed intake (g/chick)[a]						ANOVA F[(Pr > F)][e]		
	Effect of feeding			Effect of administration[d]					
	Aflatoxin[b]	Restricted[c]	Ad libitum	244	344	644	Feed[f]	Admin	Feed x Admin
1	9.68A	7.88B	9.61A	-A	-A	-A	14.8(0.0001)	1.44(0.26)	1.37(0.27)
2	20.6A	17.6B	21.1A	-A	-A	-A	20.9(0.0001)	0.52(0.60)	0.87(0.48)
3	36.5A	31.3B	37.5A	-A	-A	-A	26.6(0.0001)	0.47(0.63)	1.36(0.27)
4	55.6A	47.5B	58.1A	-A	-A	-A	18.2(0.0001)	0.62(0.55)	2.09(0.11)
5	77.5B	69.6C	84.8A	-A	-A	-A	12.7(0.0001)	0.74(0.49)	2.98(0.037)
6	100B	92.6B	110A	-A	-A	-A	9.31(0.0008)	1.62(0.22)	3.02(0.035)
7	130B	122B	143A	127B	129B	141A	8.55(0.0013)	4.25(0.025)	2.33(0.082)
8	165B	156B	180A	161B	161B	180A	8.02(0.0018)	6.44(0.0052)	2.64(0.056)
9	203B	197B	221A	202B	198B	222A	5.53(0.0097)	6.10(0.0062)	3.25(0.027)
10	245B	236B	270A	241B	240B	271A	6.35(0.0055)	6.39(0.0054)	2.64(0.056)
11	288B	280B	316A	280B	282B	321A	6.05(0.0067)	8.94(0.0001)	3.34(0.024)
12	333B	326B	368A	325B	327B	376A	6.15(0.0063)	10.32(0.0005)	3.88(0.013)
13	383B	376B	423A	373B	375B	434A	5.06(0.014)	9.18(0.0009)	3.46(0.021)
14	433B	427B	490A	427B	428B	494A	6.99(0.0036)	8.45(0.0014)	3.31(0.025)
15	484B	478B	550A	481B	481B	549A	7.46(0.0026)	7.27(0.0030)	3.13(0.031)
16	524B	518B	606A	528B	528B	591A	10.5(0.0004)	5.77(0.0082)	2.61(0.058)
17	561B	554B	663A	571B	572B	635A	15.9(0.0001)	5.81(0.0080)	2.64(0.056)
18	598B	591B	719A	614B	615B	679A	21.7(0.0001)	5.81(0.0079)	2.52(0.065)
19	640B	635B	782A	663B	663B	730A	27.3(0.0001)	5.85(0.0077)	2.34(0.080)
20	681B	676B	850A	716B	709B	782A	40.1(0.0001)	6.55(0.0048)	2.46(0.069)
21	727B	723B	920A	770B	763B	837A	50.3(0.0001)	6.67(0.0044)	2.13(0.10)
22	774B	771B	993A	826B	819B	892A	60.6(0.0001)	6.11(0.0065)	1.81(0.16)
23	822B	819B	1073A	883B	874B	957A	74.7(0.0001)	7.16(0.0032)	1.47(0.24)
24	881B	878B	1157A	954B	946B	1017A	82.3(0.0001)	4.77(0.017)	1.47(0.24)

[a]Means for feed intakes within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin = 2.5 μg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of folic acid (basal ration provided 244%).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 6. Effect of graded levels of dietary folic acid on the feed conversion of with aflatoxicosis.

Day	Aflatoxin (2.5 µg/g of diet) 244[c]	344[c]	644[c]	Restricted Feeding[b] 244	344	644	Control 244	344	644
1	1.01±0.17A	0.90±0.09A	0.88±0.09A	0.83±0.09A	0.82±0.56A	0.80±0.09A	0.96±0.21A	0.94±0.05A	0.83±0.11A
2	1.16±0.06A	1.11±0.09A	1.05±0.06A	0.90±0.07B	0.89±0.10B	0.88±0.08B	1.09±0.13A	1.10±0.1A	1.07±0.08A
3	1.26±0.02AB	1.08±0.45AB	1.23±0.08AB	1.04±0.08AB	1.03±0.1B	1.01±0.08B	1.25±0.15AB	1.35±0.15A	1.28±0.04AB
4	1.41±0.12AB	1.35±0.13B	1.46±0.20AB	1.13±0.1C	1.12±0.07C	1.08±0.04C	1.33±0.14B	1.54±0.22A	1.44±0.05AB
5	1.45±0.14AB	1.42±0.06AB	1.67±0.50A	1.23±0.1B	1.19±0.04B	1.26±0.90B	1.41±0.16AB	1.66±0.25A	1.52±0.16AB
6	1.58±0.17AB	1.43±0.02BC	1.69±0.21A	1.28±0.1CD	1.20±0.03D	1.38±0.14BCD	1.38±0.16BCD	1.78±0.24A	1.57±0.17AB
7	1.47±0.18BC	1.43±0.02BC	1.74±0.21A	1.30±0.11C	1.28±0.02C	1.46±0.07BC	1.44±0.11BC	1.77±0.26A	1.63±0.15AB
8	1.62±0.19BCD	1.51±0.04CDE	1.87±0.21A	1.38±0.11DE	1.31±0.05E	1.56±0.12CD	1.51±0.11DE	1.85±0.30AB	1.74±0.18A-C
9	1.77±0.20AB	1.57±0.07BC	2.03±0.20A	1.62±0.15BC	1.43±0.05C	1.64±0.14BC	1.58±0.09BC	1.95±0.31A	1.82±0.19AB
10	1.88±0.22AB	1.67±0.05BC	2.15±0.25A	1.66±0.24BC	1.51±0.08C	1.73±0.19BC	1.65±0.07BC	2.08±0.35A	1.90±0.17AB
11	1.90±0.23BC	1.69±0.07CDE	2.27±0.23A	1.61±0.17DE	1.43±0.02E	1.77±0.15CD	1.61±0.06DE	2.07±0.34AB	1.90±0.17BC
12	2.04±0.21BC	1.73±0.09DE	2.42±0.26A	1.67±0.15EF	1.45±0.05F	1.83±0.13CDE	1.61±0.08EF	2.13±0.32B	1.93±0.17BCD
13	2.14±0.24B	1.84±0.13CD	2.59±0.30A	1.76±0.17CDE	1.55±0.04E	1.96±0.14BC	1.63±0.10DE	2.17±0.37B	1.99±0.17BC
14	2.23±0.27BC	1.96±0.16CDE	2.71±0.35A	1.86±0.19DE	1.63±0.05E	2.06±0.18BCD	1.70±0.13E	2.23±0.38B	2.05±0.15BCD
15	2.29±0.27B	1.97±0.22BCD	2.77±0.35A	1.82±0.14DEF	1.61±0.09F	1.94±0.13CDE	1.64±0.18EF	2.24±0.37BC	2.01±0.16BCD
16	2.37±0.27B	1.95±0.24CDE	2.70±0.32A	1.79±0.03DEF	1.61±0.11F	1.85±0.17DEF	1.63±0.12EF	2.24±0.37BC	2.05±0.24BCD
17	2.26±0.24B	1.92±0.22CD	2.63±0.31A	1.71±0.12DEF	1.58±0.09F	1.86±0.15DE	1.62±0.10EF	2.14±0.26BC	1.96±0.13CD
18	2.24±0.23B	1.91±0.23CDE	2.60±0.27A	1.71±0.09EF	1.61±0.11F	1.76±0.08DEF	1.63±0.12F	2.16±0.28CB	1.98±0.12CD
19	2.30±0.23B	1.88±0.21D	2.54±0.28A	1.67±0.07DE	1.60±0.09E	1.73±0.10DE	1.62±0.05E	2.15±0.28BC	1.91±0.14CD
20	2.26±0.22B	1.87±0.17DE	2.53±0.28A	1.70±0.07DE	1.65±0.05E	1.67±0.12E	1.64±0.11E	2.14±0.27BC	1.92±0.13CD
21	2.19±0.20B	1.89±0.17C	2.48±0.27A	1.77±0.10CD	1.70±0.05CD	1.71±0.12CD	1.65±0.03D	2.19±0.31B	1.90±0.11C
22	2.19±0.20B	1.89±0.17DE	2.47±0.24A	1.79±0.10DEF	1.72±0.04EF	1.79±0.13DEF	1.65±0.07F	2.10±0.21BC	1.95±0.06CD
23	2.27±0.32AB	1.84±0.15CDE	2.37±0.36A	1.76±0.09DE	1.67±0.06E	1.76±0.11DE	1.63±0.06E	2.06±0.20BC	1.97±0.08CD
24	2.23±0.25B	1.91±0.16CD	2.48±0.27A	1.84±0.15D	1.59±0.17E	1.79±0.10DE	1.72±0.04DE	2.15±0.19B	2.07±0.06BC

Feed conversion ratio[a]

[a] Values (means ± standard deviations) for feed conversion (feed intake/weight gain) followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of folic acid (basal ration provides 244%).

App. 6a. Major and interactive effects produced by graded levels of dietary folic acid on the feed conversion of chicks with aflatoxicosis.

Day	Feed conversion ratio[a]						ANOVA (F[Pr > F])[e]		
	Effect of feeding			Effect of administration[d]					
	Aflatoxin[b]	Restricted[c]	Ad libitum	244	344	644	Feed	Admin	Feed x Admin
1	0.93A	0.82B	0.91AB	-A	-A	-A	3.16(0.059)	2.12(0.14)	0.53(0.72)
2	1.11A	0.89B	1.09A	-A	-A	-A	23.1(0.0001)	0.86(0.43)	0.38(0.82)
3	1.19A	1.03B	1.29A	-A	-A	-A	7.05(0.0034)	0.09(0.91)	0.75(0.57)
4	1.41A	1.11B	1.43A	-A	-A	-A	22.5(0.0001)	0.31(0.73)	1.48(0.24)
5	1.51A	1.23B	1.53A	-A	-A	-A	7.58(0.0024)	0.97(0.39)	1.11(0.37)
6	1.57A	1.29B	1.57A	-A	-A	-A	13.2(0.0001)	2.09(0.14)	4.37(0.0074)
7	1.55A	1.35B	1.61A	1.40B	1.50AB	1.61A	10.5(0.0004)	6.00(0.007)	3.09(0.032)
8	1.67A	1.42B	1.70A	1.50B	1.56B	1.73A	10.5(0.0004)	5.99(0.0070)	3.11(0.031)
9	1.79A	1.57B	1.79A	1.66B	1.65B	1.83A	6.41(0.0053)	4.00(0.030)	4.60(0.0058)
10	1.90A	1.63B	1.88A	1.73B	1.75AB	1.93A	6.29(0.0057)	3.33(0.051)	4.02(0.011)
11	1.95A	1.61B	1.86A	1.71B	1.73B	1.98A	1.11(0.0003)	7.81(0.0021)	5.66(0.0019)
12	2.06A	1.65C	1.89B	1.77B	1.77B	2.06A	15.3(0.0001)	9.80(0.0006)	8.40(0.0002)
13	2.19A	1.76C	1.93B	1.85B	1.85B	2.18A	13.2(0.0001)	10.2(0.0005)	6.96(0.0005)
14	2.29A	1.85B	2.00B	1.93B	1.93B	2.28A	11.2(0.0003)	9.22(0.0009)	6.17(0.0012)
15	2.34A	1.79B	1.97B	1.92B	1.94B	2.24A	17.8(0.0001)	7.45(0.0026)	6.84(0.0006)
16	2.34A	1.75C	1.88B	1.93B	1.93B	2.20A	19.8(0.0001)	5.51(0.0099)	6.85(0.0006)
17	2.27A	1.72C	1.91B	1.86B	1.88B	2.15A	24.7(0.0001)	8.00(0.0019)	7.20(0.0004)
18	2.25A	1.70C	1.92B	1.86B	1.89B	2.11A	27.2(0.0001)	6.57(0.0047)	8.04(0.0002)
19	2.21A	1.68C	1.89B	1.83B	1.88B	2.06A	26.5(0.0001)	5.42(0.011)	8.26(0.0002)
20	2.22A	1.67C	1.90B	1.87B	1.89AB	2.04A	29.3(0.0001)	3.44(0.047)	9.53(0.0001)
21	2.19A	1.72C	1.91B	2.18A	1.77C	1.90B	21.0(0.0001)	2.51(0.10)	9.16(0.0001)
22	2.18A	1.77C	1.90B	1.88B	1.91B	2.07A	23.2(0.0001)	5.51(0.0099)	8.99(0.0001)
23	2.16A	1.73C	1.89B	1.89AB	1.86B	2.03A	1.61(0.0001)	3.00(0.067)	5.98(0.0014)
24	2.21A	1.74C	1.98B	1.93B	1.89B	2.11A	24.5(0.0001)	6.67(0.0046)	8.13(0.0002)

[a]Means of feed conversion ratios (feed intake/weight gain) within a major effect followed by the same letter are not significantly different at P≤0.05.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of folic acid (basal ration provided 244%).

[e]Admin = major effect of administration. Feed = Major effect of feeding. Feed x Admin = test for interaction.

App. 7. Effect of graded levels of dietary threonine on the weight gain of chicks with aflatoxicosis

	Weight gain (g/chick)[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
Day	128[c]	155[c]	179[c]	128	155	179	128	155	179
0	36±0.96A	36±0.60AB	39±1.6A	34±1.6B	36±2.8AB	35±2.2B	35±2.2B	34±2.2B	38±2.4AB
3	54±1.5E	55±1.7ED	63±5.7AB	60±2.4BCD	67±5.9A	60±5.1B-D	63±2.9A-C	57±3.4	59±4.0B-E
6	91±37B	92±4.5B	107±9.2A	99±4.7AB	109±5.8A	104±10AB	99±15AB	104±8.6C-E	103±6.7AB
9	140±3.8B	142±12B	158±8.5AB	145±9.4B	156±5.5AB	157±14AB	156±19AB	160±15AB	168±12A
12	193±2.5D	196±13D	215±12CD	193±13D	210±9.1CD	217±34CD	229±23CB	244±16AB	255±14A
15	245±6.3F	250±17DEF	261±36DEF	249±11EF	280±9.4CDE	282±36CD	310±34BC	331±22AB	352±20A
18	321±12D	318±27D	331±29D	316±31D	346±14CD	331±32D	367±30BC	383±31B	460±20A
22	443±22D	430±40D	457±32CD	446±46D	472±15CD	495±61BC	537±21B	522±30B	647±35A
23	484±23F	465±40F	500±37EF	502±29EF	537±6.4ED	557±53CD	611±47B	603±21CB	702±42A
24	504±24EF	486±41F	519±36EF	527±55DEF	547±17DE	573±59CD	620±39CB	628±24B	729±40A

[a] Values (means ± standard deviations) for weight gain followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of threonine (basal ration provides 128%).

App. 7a. Major and interactive effects produced by graded levels of dietary threonine on the weight gain of chicks with aflatoxicosis.

Day	Weight gain (g/chick)[a]								
	Effect of feeding			Effect of administration[d]			ANOVA(F[Pr > F])[e]		
	Aflatoxin[b]	Restricted[c]	Ad libitum	128	155	179	Feed	Admin	Feed x Admin
0	36.9A	34.9B	35.4AB	34.9B	35.4AB	36.8A	3.51(0.044)	3.27(0.053)	1.58(0,21)
3	57.1B	62.3A	59.8AB	-A	-A	-A	5.14(0.013)	0.66(0.53)	6.27(0.0011)
6	96.6A	104A	102A	96.4B	102AB	105A	2.64(0.089)	3.06(0.063)	1.69(0.18)
9	147B	153AB	162A	147B	153AB	161A	14.67(0.017)	4.34(0.023)	0.49(0.75)
12	201B	206B	243A	205B	217AB	229A	20.3(0.0001)	5.69(0.0088)	0.25(0.91)
15	252B	270B	331A	268B	287AB	299A	35.6(0.0001)	4.91(0.015)	0.51(0.73)
18	323B	331B	403A	335B	349B	373A	34.1(0.0001)	6.90(0.0038)	4.56(0.0060)
22	443C	471B	569A	475B	476Ba	553A	39.9(0.0001)	10.3(0.0005)	3.14(0.03)
23	483C	532B	639A	532A	535B	586A	59.1(0.0001)	8.70(0.0012)	2.06(0.11)
24	503C	549B	659A	551A	554B	607A	49.7(0.0001)	7.79(0.0021)	1.88(0.14)

[a]Means for the gain in weights within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary reuqirement (1977) of the threonine (basal ration provided 128%).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 8. Effect of graded levels of dietary threonine on the feed intake of chicks with aflatoxicosis

	Feed intake (g/chick)[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
Day	128[c]	155[c]	179[c]	128	155	179	128	155	179
1	5.6±1.0CD	5.5±0.62D	5.9±0.51CD	7.8±1.3AB	8.2±1.5A	7.2±1.3ABC	6.5±0.61B-D	6.0±0.37CD	6.6±1.3B-D
2	15±1.2CD	14±0.77D	16±2.1BCD	18±1.7AB	19±2.2A	17±1.2ABC	17±0.60A-D	15±2.1CD	16±1.6A-D
3	2.5±1.2DE	25±1.1E	27±2.5CDE	30±2.1AB	32±2.3A	28±1.9BC	29±0.85BC	26±1.7C-E	28±1.9DC
4	39±3.2CD	38±2.7D	43±4.7BC	47±2.9AB	48±3.0A	43±2.3BC	43±1.9BC	42±1.6CD	43±2.1BC
5	54±5.3DE	53±3.2E	61±6.9C	67±4.6AB	72±3.8A	64±2.7BC	60±5.5CD	61±2.1C	63±2.5BC
6	74±6.0BC	72±4.0C	85±9.7A	83±3.8AB	87±4.7A	83±9.4A-C	81±9.4A-C	84±2.9A	83±2.7AB
7	96±7.9BC	94±6.7C	109±11A	102±4.0A-C	106±4.4A-C	106±6.1ABC	107±11AB	109±5.7A	109±4.0A
8	120±8.7AB	118±9.5B	136±14A	124±5.0AB	127±5.6AB	132±9.0AB	132±13AB	133±9.2AB	134±4.5AB
9	148±9.4B	147±13B	168±17A	148±7.1B	152±7.5AB	159±12AB	163±15AB	170±9.5A	170±4.7A
10	173±13D	172±14D	197±23ABC	176±84CD	184±7.1BCD	191±15A-D	193±17A-D	207±12A	201±5.4AB
11	203±18CD	200±15D	230±28AB	201±11CD	208±7.7BCD	220±21A-D	228±22ABC	246±13A	237±7.1A
12	235±24ED	242±16CDE	267±37A-D	230±15E	237±8.0ED	255±25B-E	269±26ABC	293±14A	281±8.8AB
13	276±29CDE	258±25E	303±48BCD	263±21ED	270±7.3ED	292±33B-E	313±30ABC	346±22A	328±11AB
14	313±35D	298±23D	341±54BCD	296±26D	303±4.6D	326±41CD	363±36ABC	401±28A	381±13AB
15	352±38C	337±21C	379±64BC	332±31C	343±5.0C	362±45BC	409±40AB	453±30A	433±14A
16	401±48CD	387±20CD	435±74BC	371±36D	383±4.6CD	402±56CD	469±45AB	521±29A	505±20A
17	439±54C	424±18C	471±81BC	420±45C	435±3.8C	458±66BC	513±50AB	545±9.5A	558±21A
18	490±59BC	474±19C	514±85BC	458±50C	472±9.8C	490±68BC	560±56AB	599±14A	619±22A
19	543±66C	527±25C	566±99BC	509±55C	521±17C	538±77C	626±56AB	668±17A	686±29A
20	603±72C	585±34C	618±104BC	563±62C	575±24C	589±79C	698±58AB	735±22A	758±40A
21	665±78B	645±41B	674±109B	622±67B	629±32B	642±85B	777±67A	806±30A	842±53A
22	736±84B	719±49B	737±111B	685±73B	693±34B	698±91B	855±72A	878±25A	931±58A
23	792±88B	778±57B	793±106B	755±78B	767±43B	762±94B	920±70A	938±35A	998±66A
24	864±91B	853±69B	863±113B	811±82B	825±50B	809±100B	1004±67A	1029±43A	1084±79A

[a] Values (means ± standard deviations) for feed intake followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of threonine (basal ration provides 128%).

App. 8a. Major and interactive effects produced by graded levels of dietary threonine on the feed intake of chicks with aflatoxicosis.

Day	Feed intake (g/chick)[a]						ANOVA (F(Pr > F))[e]		
	Effect of feeding			Effect of administration[d]					
	Aflatoxin[b]	Restricted[c]	Ad libitum	128	255	179	Feed	Admin	Feed x Admin
1	5.64B	7.72A	6.36B	-A	-A	-A	12.9(0.0001)	0.02(0.98)	0.84(0.51)
2	15.0B	17.9A	15.9B	-A	-A	-A	10.3(0.0005)	0.74(0.48)	1.51(0.23)
3	25.5C	30.0A	27.6B	-A	-A	-A	19.0(0.0001)	0.47(0.63)	2.93(0.039)
4	40.0C	46.0A	42.6B	-A	-A	-A	13.3(0.0001)	0.13(0.88)	2.87(0.042)
5	56.3C	67.8A	61.3B	-A	-A	-A	21.2(0.0001)	0.71(0.50)	3.16(0.030)
6	76.8AB	84.1A	82.4A	-A	-A	-A	5.21(0.012)	1.68(0.21)	2.41(0.074)
7	99.6B	105AB	108A	102A	103A	108A	4.26(0.025)	2.78(0.080)	1.59(0.21)
8	125B	128AB	133A	125B	126AB	134A	2.67(0.087)	3.18(0.058)	1.12(0.37)
9	154B	153B	167A	153B	156B	165A	6.33(0.0058)	3.83(0.034)	0.92(0.45)
10	181B	183B	201A	181B	188AB	196A	7.46(0.0026)	3.91(0.032)	1.33(0.29)
11	211B	210B	237A	210B	218AB	229A	9.51(0.0007)	3.64(0.040)	1.28(0.30)
12	248B	241B	281A	245B	257B	268A	12.5(0.0001)	3.51(0.044)	0.90(0.48)
13	279B	276B	329A	-A	-A	-A	14.4(0.0001)	2.30(0.12)	1.54(0.22)
14	317B	308B	381A	-A	-A	-A	18.5(0.0001)	1.92(0.16)	1.13(0.36)
15	356B	346B	432A	-A	-A	-A	20.5(0.0001)	1.68(0.20)	0.93(0.46)
16	408B	385B	498A	-A	-A	-A	24.2(0.0001)	1.95(0.16)	0.80(0.54)
17	445B	438B	539A	-A	-A	-A	18.1(0.0001)	2.18(0.13)	0.28(0.89)
18	493B	473B	593A	-A	-A	-A	20.2(0.0001)	1.89(0.17)	0.34(0.85)
19	545B	523B	660A	-A	-A	-A	20.8(0.0001)	1.35(0.28)	0.30(0.87)
20	602B	575B	730A	-A	-A	-A	22.3(0.0001)	0.99(0.38)	0.25(0.91)
21	662B	631B	808A	-A	-A	-A	23.9(0.0001)	0.73(0.49)	0.24(0.91)
22	731B	692B	888A	-A	-A	-A	25.5(0.0001)	0.61(0.55)	0.35(0.84)
23	787B	761B	952A	-A	-A	-A	23.4(0.0001)	0.50(0.61)	0.39(0.81)
24	860B	815B	1039A	-A	-A	-A	26.3(0.0001)	0.31(0.73)	0.40(0.81)

[a]Means for feed intakes within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of threonine (basal ration provided 128%).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 9. Effect of graded levels of dietary threonine on the feed conversion of chicks with aflatoxicosis

	Feed conversion ratio[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
Day	128[c]	155[c]	179[c]	122	155	179	128	155	179
3	1.42±0.06A	1.35±0.15AB	1.10±0.13CD	1.17±0.11BCD	1.0±0.09D	1.13±0.08CD	1.02±0.12D	1.18±0.28CD	1.28±0.20ABC
6	1.34±0.08A	1.30±0.13A	1.24±0.09A	1.27±0.07A	1.18±0.01A	1.20±0.13A	1.29±0.25A	1.22±0.14A	1.26±0.09A
9	1.43±0.09A	1.41±0.23A	1.40±0.08A	1.35±0.08A	1.26±0.05A	1.30±0.08A	1.35±0.17A	1.36±0.17A	1.30±0.10A
12	1.50±0.16A	1.52±0.12A	1.51±0.11A	1.46±0.08A	1.36±0.06A	1.42±0.15A	1.39±0.14A	1.41±0.15A	1.29±0.06A
15	1.68±0.21A	1.59±0.22AB	1.71±0.13A	1.55±0.09AB	1.41±0.05B	1.47±0.08AB	1.49±0.12AB	1.54±0.19AB	1.38±0.04B
18	1.72±0.19AB	1.69±0.17AB	1.76±0.20A	1.63±0.08AB	1.53±0.09AB	1.65±0.14AB	1.68±0.07AB	1.73±0.18AB	1.47±0.06B
22	1.81±0.19A	1.84±0.15A	1.75±0.17AB	1.67±0.07ABC	1.59±0.07BC	1.52±0.09C	1.70±0.14ABC	1.80±0.08A	1.53±0.06C
23	1.76±0.16AB	1.82±0.14A	1.71±0.12ABC	1.61±0.10B-E	1.53±0.07DE	1.46±0.07E	1.60±0.12CDE	1.65±0.06BCD	1.50±0.09DE
24	1.84±0.17AB	1.90±0.14A	1.79±0.13ABC	1.65±0.07C-F	1.61±0.06DEF	1.50±0.08F	1.72±0.15B-E	1.73±0.04BCD	1.57±0.08EF

[a] Values (means ± standard deviations) for weight gain followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of threonine (basal ration provides 128%).

App. 9a. Major and interactive effects produced by graded levels of dietary threonine on the feed conversion of chicks with aflatoxicosis.

Day	Feed conversion ratio[a]						ANOVA (F(Pr > F))[e]		
	Effect of feeding			Effect of administration[d]					
	Aflatoxin[b]	Restricted[c]	Ad libitum	128	155	179	Feed	Admin	Feed x Admin
3	1.29A	1.11B	1.16B	-A	-A	-A	5.80(0.0081)	0.19(0.83)	5.59(0.0021)
6	-A	-A	-A	-A	-A	-A	1.05(0.36)	1.17(0.33)	0.21(0.93)
9	-A	-A	-A	-A	-A	-A	2.33(0.12)	0.36(0.70)	0.21(0.93)
12	1.51A	1.41AB	1.36B	-A	-A	-A	4.51(0.020)	0.34(0.71)	0.67(0.62)
15	1.66A	1.48B	1.47B	-A	-A	-A	7.18(0.0032)	0.72(0.50)	1.17(0.35)
18	-A	-A	-A	-A	-A	-A	2.45(0.11)	0.43(0.66)	2.37(0.078)
22	1.80A	1.59B	1.08B	1.73A	1.74A	1.60B	8.76(0.0012)	4.80(0.017)	1.12(0.37)
23	1.77A	1.53B	1.59B	1.66A	1.67A	1.56B	15.5(0.0001)	4.92(0.015)	0.33(0.69)
24	1.85A	1.59B	1.67B	1.74A	1.75A	1.62B	16.8(0.0001)	4.92(0.015)	0.33(0.86)

[a]Means of feed conversion ratios (feed intake/weight gain) within a major effect followed by the same letter are not significantly different at $P<0.05$.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the feed intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of threonine (basal ration provided 128%).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 10. Effect of graded levels of dietary lysine on the weight gain of chicks with aflatoxicosis

	Weight gain (g/chick)[a]								
	Aflatoxin (2.5 µg/g of diet)			Restricted Feeding[b]			Control		
Day	102[c]	122[c]	146[c]	102	122	146	102	122	146
0	37.6±0.58A	38.1±2.6A	37.9±0.50A	38.5±2.3A	37.7±0.26A	36.9±1.6A	37.1±2.7A	39.1±0.87A	37.2±2.5A
3	51.6±2.6AB	53.4±1.5A	53.0±3.6AB	50.4±1.9AB	52.7±2.3AB	48.6±3.6B	51.1±2.8AB	55.0±3.2A	53.3±0.46AB
6	74.4±2.1C	80.4±3.4BC	81.3±2.2BC	81.1±4.7BC	84.3±6.6AB	75.4±8.9C	79.5±5.4BC	90.2±6.3A	81.3±1.3BC
9	113±3.0C	124±3.2B	124±5.8B	130±5.0AB	130±8.1AB	127±8.8B	129±7.4B	140±9.3A	129±0.66B
12	155±3.8E	171±5.3D	175±8.5CD	194±7.3AB	189±13B	187±13B	187±11BC	207±12A	191±7.0B
15	205±8.2D	224±11CD	227±11C	277±10AB	266±19B	260±21B	280±26AB	293±13A	268±9.3B
18	252±14E	272±18DE	277±13D	352±3.6B	337±20BC	318±27C	340±25BC	382±18A	341±20BC
23	351±26E	394±24D	388±20D	457±35C	446±11C	436±5.7B	518±42B	558±23A	521±20B
24	367±32F	411±25DE	402±20EF	461±33C	446±13CD	448±11C	545±47B	586±22A	546±21B

[a] Values (means ± standard deviations) for weight gain followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of lysine (basal ration provides 102%).

App. 10a. Major and interactive effects produced by graded levels of dietary lysine on the weight gain of chicks with aflatoxicosis.

Day	Weight gain (g/chick)[a]						ANOVA (F[Pr > F])[e]		
	Effect of feeding			Effect of administration[d]					
	Aflatoxin[b]	Restricted[c]	Ad libitum	102	122	146	Feed	Admin	Feed x Admin
0							0.03(0.97)	0.77(0.47)	0.76(0.56)
3	52.7AB	50.6B	53.1A	51.0B	53.7A	51.6B	3.24(0.055)	3.46(0.046)	0.87(0.48)
6	78.7B	80.3AB	83.7A	78.4B	85.0A	79.3B	2.94(0.070)	5.85(0.0078)	2.21(0.095)
9	120B	129A	133A	124B	132A	126AB	10.7(0.0004)	4.20(0.026)	1.92(0.14)
12	167B	190A	195A	179B	189A	184AB	28.5(0.0001)	3.40(0.048)	3.29(0.026)
15	219C	268B	280A	-A	-A	-A	53.7(0.0001)	1.25(0.30)	2.62(0.057)
18	267C	336B	354A	315AB	330A	312B	73.5(0.0001)	3.36(0.050)	4.40(0.0072)
23	378C	446B	532A	442B	466A	448AB	113(0.0001)	2.93(0.071)	2.13(0.10)
24	393C	452B	559A	-A	-A	-A	117(0.0001)	2.23(0.13)	2.03(0.12)

[a]Means for the gain in weights within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of lysine (basal ration provided 102%).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 11 Effect of graded levels of dietary lysine on the feed intake of chicks with aflatoxicosis.

	Feed intake (g/chick)[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
Day	102[c]	122[c]	146[c]	102	122	146	102	122	146
1	10.3±4.7A	3.93±0.72E	4.76±1.3DE	7.94±3.3A-D	5.59±1.2CDE	7.06±2.1A-E	8.94±3.3ABC	9.56±1.6AB	6.47±2.4B-E
2	18.9±2.8A	13.5±0.30D	9.11±4.7E	13.7±2.1CD	11.9±1.4DE	13.1±3.1DE	18.0±0.30AB	17.7±3.9ABC	14.0±0.46BCD
3	33.1±4.3A	25.9±4.7BCD	20.7±3.6D	23.3±2.6CD	23.0±1.3CD	25.7±6.0BCD	29.4±2.4AB	32.1±4.8A	28.9±3.7ABC
4	53.0±9.0AB	41.4±9.3CDE	32.4±6.9E	35.6±3.4DE	38.7±2.1CDE	42.5±9.5CD	45.7±4.7BC	59.7±5.4A	44.8±6.5BCD
5	73.3±9.4AB	60.2±11CD	49.7±9.0D	54.2±4.8CD	60.0±5.2CD	60.1±14CD	62.7±5.5BC	82.1±6.7A	63.5±7.9BC
6	97.7±13AB	82.0±13C	71.6±12C	74.6±6.3C	80.2±6.3C	77.0±12C	83.2±7.4BC	104±8.8A	86.0±9.9BC
7	126±14B	110±15BC	101±17C	99.0±8.4C	102±8.0C	102±12C	115±13BC	147±13A	112±12BC
8	158±16B	142±18BCD	134±21CD	128±9.2D	130±11CD	132±15CD	150±11BC	182±16A	142±12BCD
9	200±19B	181±18BC	179±26BC	160±9.2C	162±11C	168±15C	196±9.1B	237±21A	178±14BC
10	240±24B	220±18BC	220±33BC	201±9.2C	201±11C	218±15BC	241±16B	289±26A	213±17BC
11	263±22BC	251±21BC	253±33BC	241±9.2C	240±11C	256±15BC	273±17B	315±27A	251±20BC
12	296±28BC	287±21BC	289±38BC	264±9.2C	273±11C	291±15BC	320±17B	370±30A	290±23BC
13	342±28BC	328±21CD	332±42CD	300±9.2D	309±11CD	325±15CD	373±17B	434±33A	341±29BC
14	375±31C	362±21C	366±44C	346±9.2C	351±11C	367±15C	418±14B	487±37A	380±30C
15	413±36C	403±23C	409±47C	398±9.2C	407±11C	407±15C	477±11B	554±40A	433±32C
16	449±41CD	442±23CD	449±51CD	439±5.5D	448±11CD	450±15CD	535±10B	621±45A	483±35C
17	487±46CD	481±24D	488±54CD	475±5.5D	490±11CD	492±15CD	508±11B	684±50A	532±36C
18	533±50D	523±26D	521±48D	513±5.5D	533±11D	533±15D	648±12B	748±53A	584±36C
19	573±49D	568±25D	563±47D	561±5.0D	575±11D	565±15D	713±14B	811±55A	646±35C
20	624±54D	620±32D	614±49D	601±8.4D	621±11D	604±15D	784±19B	893±58A	714±36C
21	675±54D	672±35D	665±56D	653±8.4D	672±11D	662±11D	842±20B	958±61A	776±37C
22	732±59D	725±39D	720±58D	700±7.0D	718±11D	709±11D	909±19B	1040±65A	846±38C
23	792±65D	783±46D	774±56D	756±8.4D	770±11D	765±11D	981±20B	1113±68A	917±40C
24	839±61D	830±52D	821±56D	817±8.4D	829±11D	818±11D	1050±22B	1180±69A	981±37C

[a] Values (means ± standard deviations) for feed intake followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of lysine (basal ration provides 102%).

App. IIa. Major and interactive effects produced by graded levels of dietary lysine on the feed intake of chicks with aflatoxicosis.

Day	Feed intake (g/chick)[a]						ANOVA (F[Pr > F])[e]		
	Effect of feeding			Effect of administration[d]					
	Aflatoxin[b]	Restricted[c]	Ad libitum	102	122	146	Feed	Admin	Feed x Admin
1				9.02A	6.36B	6.09B	2.26(0.12)	5.71(0.0086)	2.82(0.045)
2	13.9B	12.9B	16.6A	16.9A	14.4B	12.1C	5.62(0.0091)	9.01(0.0010)	3.24(0.027)
3	26.6B	24.0B	30.1A	-A	-A	-A	7.18(0.0032)	2.34(0.12)	4.41(0.0071)
4	42.2B	38.9B	50.1A	44.7AB	46.6A	39.9B	8.50(0.0014)	3.14(0.060)	6.59(0.0008)
5	61.1B	58.0B	69.5A	63.4AB	67.3AB	57.8A	5.64(0.0090)	3.68(0.039)	5.40(0.0025)
6	83.8AB	77.3B	90.9A	85.2A	88.6A	78.2A	5.46(0.010)	3.31(0.052)	4.27(0.0084)
7	112B	101C	125A	114AB	120A	105B	10.5(0.0004)	4.22(0.025)	4.88(0.0043)
8	145B	130C	158A	145AB	152A	136B	11.2(0.0003)	3.37(0.049)	3.86(0.013)
9	187B	163C	204A	185AB	194A	175B	17.2(0.0001)	3.63(0.040)	5.61(0.0020)
10	227B	207C	248A	228AB	237A	217B	12.9(0.0001)	3.06(0.064)	6.98(0.0005)
11	256B	246B	280A	-A	-A	-A	8.34(0.0015)	1.66(0.21)	4.62(0.0057)
12	291B	276B	320A	-A	-A	-A	15.2(0.0001)	2.53(0.098)	5.62(0.0020)
13	334B	311C	383A	388AB	357A	333B	25.9(0.0001)	3.21(0.056)	6.40(0.0009)
14	368B	355B	428A	379AB	400A	371B	26.6(0.0001)	3.89(0.033)	7.15(0.0005)
15	409B	404B	488A	430B	455A	416B	33.5(0.0001)	5.72(0.0085)	6.65(0.0007)
16	447B	446B	547A	475B	504A	461B	42.3(0.0001)	6.19(0.0061)	7.30(0.0004)
17	485B	486B	601A	517B	552A	504B	49.0(0.0001)	6.76(0.0042)	7.62(0.0003)
18	526B	527B	660A	565B	601A	546B	64.0(0.0001)	8.45(0.0014)	8.19(0.0002)
19	568B	567B	723A	615B	652A	591B	87.3(0.0001)	10.0(0.0006)	7.63(0.0003)
20	619B	609B	797A	670B	711A	644B	102(0.0001)	10.5(0.0004)	7.35(0.0004)
21	671B	662B	859A	723B	767A	701B	104(0.0001)	9.69(0.0007)	7.37(0.0004)
22	726B	709B	930A	781B	826A	758B	112(0.0001)	8.86(0.0011)	7.28(0.0004)
23	783B	764B	1004A	844B	889A	819B	116(0.0001)	8.32(0.0015)	6.87(0.0006)
24	830B	821B	1069A	901B	946A	873B	129(0.0001)	8.87(0.0011)	6.90(0.0006)

[a] Means for feed intakes within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Dietary aflatoxin = 2.5μg per g of diet.

[c] Pair-fed to the intake of the corresponding aflatoxin group.

[d] Percentage of the NRC dietary requirement (1977) of lysine (basal ration provided 102%).

[e] Admin = major effect of administration; Feed = major effect of feeding; Feed x Admin = test for interaction.

App. [illegible] Effect of graded levels of dietary lysine on the feed conversion of chicks with aflatoxicosis.

	Feed conversion (g/chick)[a]								
	Aflatoxin (2.5 µg/g of diet)			Restricted Feeding[b]			Control		
Day	102[c]	122[c]	146[c]	102	122	146	102	122	146
3	2.42±0.44A	1.76±0.48ABC	1.39±0.08C	2.06±0.01ABC	1.56±0.25BC	2.35±0.64AB	2.19±0.59ABC	2.08±0.46ABC	1.84±0.46ABC
6	2.65±0.36A	1.95±0.21BC	1.64±0.20C	1.75±0.02BC	1.73±0.12BC	2.03±0.34B	1.97±0.17BC	2.04±0.22B	1.97±0.35BC
9	2.66±0.32A	2.10±0.17BC	2.09±0.30BC	1.74±0.08D	1.76±0.16D	1.88±0.16CD	2.15±0.20BC	2.35±0.27B	1.94±0.17CD
12	2.52±0.15A	2.16±0.16BC	2.12±0.27BCD	1.70±0.06E	1.81±0.13E	1.94±0.14CDE	2.13±0.12BCD	2.21±0.25B	1.90±0.22DE
15	2.46±0.10A	2.17±0.14B	2.17±0.27B	1.67±0.06D	1.79±0.11CD	1.84±0.17CD	1.98±0.20BC	2.18±0.17B	1.89±0.20CD
18	2.49±0.09A	2.25±0.13B	2.18±0.25B	1.63±0.04E	1.78±0.09DE	1.91±0.19D	2.15±0.13BC	2.18±0.16B	1.93±0.21CD
23	2.52±0.09A	2.20±0.11B	2.21±0.23B	1.82±0.17D	1.89±0.03CD	1.92±0.06CD	2.05±0.13BC	2.15±0.13B	1.90±0.12CD
24	2.55±0.11A	2.23±0.08BC	2.26±0.21B	1.94± 0.17E	2.03±0.06DE	1.99±0.08DE	2.07±0.14CDE	2.16±0.13BCD	1.93±0.11E

[a] Values (means ± standard deviations) for feed conversion (feed intake/weight gain followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of lysine (basal ration provides 102%).

App. 12a. Major and interactive effects produced by graded levels of dietary lysine on the feed conversion of chicks with aflatoxicosis.

Day	Feed conversion (g/chick)[a]						ANOVA (F(Pr > F))[e]		
	Effect of feeding			Effect of administration[d]					
	Aflatoxin[b]	Restricted[c]	Ad libitum	102	122	146	Feed	Admin	Feed x Admin
3				222A	1.80A	1.86A	0.46(0.64)	2.77(0.081)	2.69(0.053)
6	2.08A	1.84B	1.99AB	21.2A	1.91B	1.88B	3.11(0.061)	3.62(0.041)	8.27(0.0002)
9	2.29A	1.79B	2.15A	2.18A	2.07AB	1.97B	16.8(0.0001)	2.91(0.072)	5.20(0.0031)
12	2.27A	1.82C	2.08B	-A	-A	-A	20.0(0.0001)	1.65(0.21)	4.81(0.0046)
15	2.27A	1.76C	2.02B	-A	-A	-A	26.2(0.0001)	0.95(0.42)	3.61(0.018)
18	2.31A	1.78C	2.09B	-A	-A	-A	34.7(0.0001)	0.84(0.44)	4.78(0.0048)
23	2.32A	1.88C	2.03B	2.13B	2.08A	2.01A	34.7(0.0001)	2.35(0.097)	4.54(0.0062)
24	2.35A	1.99B	2.05B	2.19A	2.14AB	2.06B	25.9(0.0001)	2.96(0.069)	4.14(0.0096)

[a]Means of feed conversion ratio (feed intake/weight gain) within a major effect followed by the same letter are not significantly different at P≤0.05.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the feed intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of lysine (basal provided 102%).

[e]Admin = major effect of administrations. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 13. Effect of graded levels of lysine and arginine on feed intake of chicks with aflatoxicosis.[a]

Day	Aflatoxin (2.5μg/g of diet)				Restricted Feeding[b]				Ad Libitum Control			
	94 Arg[c]	122 Arg			94 Arg	122 Arg			94 Lys	122 Arg		
	102 Lys[c]	102 Lys	122 Lys	146 Lys	102 Lys	102 Lys	122 Lys	146 Lys	102 Lys	102 Lys	122 Lys	146 Lys
2	18.5±0.8A	19.5±2.2A	18.3±2.1A	18.3±1.9A	19.1±2.0A	17.7±1.4A	19.3±0.6A	17.8±1.6A	21.6±1.5A	17.6±2.4A	20.0±3.8A	19.2±0.3A
3	33.0±1.6AB	35.8±3.4AB	32.7±2.5AB	32.5±2.0B	32.7±1.7AB	32.1±1.0B	34.6±1.2AB	31.3±2.4B	37.9±2.7A	32.5±2.0B	34.8±5.4AB	33.8±1.4AB
4	51.9±2.1AB	53.8±4.6AB	51.4±2.8AB	50.7±3.6AB	49.2±1.6AB	51.5±1.7AB	53.4±2.6AB	47.9±3.3B	55.9±3.8A	50.1±4.0AB	52.5±6.7AB	50.6±2.7AB
5	73.9±2.3AB	73.6±6.3AB	70.2±2.0B	70.7±3.6B	70.2±2.5B	78.1±4.8AB	79.0±6.4AB	70.5±5.1B	81.1±7.8A	72.6±5.8AB	77.0±9.3AB	72.3±4.1AB
6	99.0±3.1AB	95.6±8.5AB	91.4±2.6B	94.2±3.7AB	92.3±2.5B	97.9±4.8AB	97.9±6.4AB	90.5±5.1B	105±9.3A	99.9±6.2AB	101±13AB	95±5.5AB
7	127±5.3ABC	120±9.7ABC	116±3.2BC	121±5.9ABC	117±2.5ABC	120±4.8ABC	119±6.4ABC	114±5.1C	133±14A	127±8.1ABC	131±19AB	123±8.9ABC
8	152±6.5AB	147±10AB	141±4.0B	149±5.9AB	145±2.5AB	145±57AB	150±17AB	141±5.1B	161±15AB	161±9.7AB	164±21A	153±14AB
9	175±5.7A	172±8.3A	167±6.7A	177±7.9A	170±2.5A	165±12A	175±17A	170±5.1A	181±6.4A	184±12A	189±27A	178±11A
10	201±7.0BC	200±6.3BC	196.9±8.4BC	203±11ABC	197±2.5BC	194±10C	207±17ABC	203±7.3ABC	211±12ABC	220±16AB	226±30A	211±12ABC
11	239±6.8BCD	231±7.4CD	228±11CD	237±11BCD	223±2.5D	221±9.9D	238±17BCD	230±7.3CD	247±7.9ABC	257±18AB	262±33A	249±13ABC
12	266±13BC	254±8.4C	254±13C	266±13BC	262±2.5C	251±9.9C	269±17BC	263±7.3C	287±10AB	294±17A	300±36A	287±13ABC
13	302±14BCD	292±11CD	292±16CD	302±16BC	299±2.5CD	278±9.9D	299±17CD	292±7.3CD	328±14A	337±22A	343±38A	325±15AB
14	340±9.7B	326±12BC	328±19BC	337±17BC	325±2.5BC	313±9.9C	333±17BC	329±7.3BC	371±16A	381±23A	388±42A	369±24A
15	375±14B	360±12BC	362±24BC	369±14BC	362±2.5BC	344±8.3C	364±17BC	363±7.3BC	413±18A	425±27A	433±47A	409±18A
16	422±19C	405±13CD	404±27CD	411±15C	390±2.5CD	378±8.3D	403±17CD	395±7.3CD	469±22AB	479±29AB	487±51A	456±20B
17	464±25B	446±26BC	454±32BC	451±25BC	448±7.9BC	423±8.3C	445±17BC	438±7.3BC	523±27A	532±26A	539±50A	510±18A
18	516±25B	492±31BC	500±36BC	495±28BC	494±7.9BC	464±8.3C	494±17BC	477±7.3C	581±24A	593±32A	598±55A	572±23A
19	562±26B	537±32BC	545±44BC	540±32BC	542±7.9BC	510±8.3C	540±17BC	521±7.3BC	633±24A	651±37A	655±63A	630±26A
20	614±23B	575±35BC	589±51BC	583±35BC	587±7.8BC	555±8.3C	586±17BC	566±7.3C	692±27A	711±38A	709±71A	686±29A
21	671±25B	620±37C	638±57BC	630±38BC	640±7.8BC	593±8.3C	630±17BC	609±7.3C	757±25A	770±38A	765±75A	742±31A
22	742±27B	677±39CD	698±60BC	687±41CD	696±7.9BC	639±8.3D	679±24CD	657±7.3CD	830±35A	843±43A	879±83A	815±34A
23	803±24B	731±44CD	754±75BCD	739±43CD	760±7.8BC	696±8.3D	740±34CD	713±7.3CD	901±34A	907±46A	891±89A	878±36A
24	864±22B	793±53CD	817±85BCD	794±46CD	828±7.8BC	752±8.3D	796±34CD	765±7.3CD	977±31A	976±51A	960±96A	949±39A
25	925±18B	848±54CD	876±96BCD	847±46CD	899±7.8BC	812±8.3D	858±34BCD	820±7.3D	1052±38A	1047±55A	1026±100A	1019±43A
26	980±22B	898±56CD	930±104BCD	892±48CD	951±7.8BC	867±8.3D	918±34BCD	873±7.3D	1119±41A	1107±60A	1084±105A	1080±43A

[a]Values (means ± standard deviations) for feed intake followed by the same letter are not significantly different at $P \leq 0.05$. Initial number of 24 chicks per treatment was reduced to 20 chicks at 7 days.
[b]Pair fed to the feed intake of the corresponding aflatoxin group.
[c]Percentage of the NRC dietary requirement (1977) of lysine or arginine (basal ration provides 94% and 102%, respectively).

App. 13a. Major and interactive effects produced by graded levels of dietary lysine and arginine on feed intake of chicks with aflatoxicosis.

Day	Feed intake (g/chick)[a]							ANOVA (F[Pr > F])[e]		
	Effect of feeding			Effect of administration[d]						
	Aflatoxin[b]	Restricted[c]	Ad libitum	94Arg+102Lys	122Arg+102Lys	122Arg+122Lys	122Arg+146Lys	Feed	Admin	Feed x Admin
2	-A	-A	-A	-A	-A	-A	-A	1.54(0.23)	1.48(0.24)	1.27(0.30)
3	-A	-A	-A	-A	-A	-A	-A	2.12(0.14)	1.05(0.38)	2.01(0.090)
4	-A	-A	-A	-A	-A	-A	-A	1.11(0.34)	1.45(0.24)	1.53(0.20)
5	-A	-A	-A	-A	-A	-A	-A	1.79(0.18)	1.56(0.22)	2.15(0.071)
6	95.1B	94.7B	101A	-A	-A	-A	-A	3.90(0.029)	1.60(0.21)	1.12(0.37)
7	121B	118B	128A	-A	-A	-A	-A	6.13(0.0051)	1.03(0.39)	0.74(0.62)
8	147B	145B	159A	-A	-A	-A	-A	7.42(0.0020)	0.42(0.74)	0.61(0.72)
9	173B	170B	183A	-A	-A	-A	-A	5.32(0.0095)	0.16(0.92)	0.68(0.67)
10	200B	200B	217A	-A	-A	-A	-A	8.71(0.0008)	0.57(0.64)	0.75(0.61)
11	234B	228B	254A	-A	-A	-A	-A	15.0(0.0001)	0.60(0.62)	1.04(0.42)
12	262B	261B	292A	-A	-A	-A	-A	19.8(0.0001)	0.53(0.66)	0.69(0.66)
13	297B	289B	333A	-A	-A	-A	-A	29.1(0.0001)	0.59(0.63)	0.92(0.49)
14	332B	325B	377A	-A	-A	-A	-A	38.6(0.0001)	0.55(0.65)	0.94(0.48)
15	366B	359B	420A	-A	-A	-A	-A	42.0(0.0001)	0.70(0.56)	0.95(0.47)
16	411B	393C	473A	-A	-A	-A	-A	53.6(0.0001)	0.74(0.53)	1.01(0.44)
17	455B	438B	526A	-A	-A	-A	-A	54.6(0.0001)	1.02(0.40)	0.62(0.72)
18	501B	482C	586A	-A	-A	-A	-A	63.9(0.0001)	1.20(0.33)	0.62(0.71)
19	546B	528B	642A	-A	-A	-A	-A	62.1(0.0001)	0.91(0.45)	0.61(0.72)
20	590B	574B	699A	-A	-A	-A	-A	64.0(0.0001)	1.00(0.40)	0.66(0.68)
21	640B	618B	760A	-A	-A	-A	-A	71.1(0.0001)	1.64(0.20)	0.71(0.64)
22	701B	668C	831A	759A	719B	735AB	720B	71.2(0.0001)	2.50(0.075)	0.66(0.68)
23	757B	729B	894A	824A	779B	793AB	777B	62.8(0.0001)	2.88(0.049)	0.73(0.63)
24	817B	785C	966A	890A	841B	836AB	834B	62.9(0.0001)	3.02(0.042)	0.56(0.76)
25	874B	845B	1036	956A	902B	920AB	985B	63.4(0.0001)	3.26(0.032)	0.54(0.78)
26	925B	902B	1096A	1015A	957B	977AB	948B	60.3(0.0001)	3.58(0.023)	0.56(0.76)

[a]Means for feed intakes within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the feed intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of lysine or arginine (basal ration provided 102% and 94%, respectively).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 14: Effect of graded levels of lysine and arginine on weight gain of chicks with aflatoxicosis[a]

Day	Aflatoxin (2.5 µg/g of diet) 94 Arg	122 Arg			Restricted Feeding[b] 94 Arg	122 Arg			Ad Libitum Control 94 Arg	122 Arg		
	102 Lys[c]	102 Lys	122 Lys	146 Lys	102 Lys	102 Lys	122 Lys	146 Lys	102 Lys	102 Lys	122 Lys	146 Lys
0	45.3±0.8A	44.6±0.8A	44.6±0.6A	44.9±0.9A	44.8±0.4A	45.0±0.6A	45.3±0.5A	45.2±0.6A	44.8±0.7A	44.9±0.5A	44.8±0.4A	44.3±0.8A
3	71.5±2.1A	72.0±3.0A	70.0±1.3A	69.2±3.6A	69.7±1.8A	70.9±5.2A	68.4±1.0A	69.6±1.4A	71.1±7.2A	69.5±1.3A	66.6±2.9A	70.3±2.1A
6	110±4.1A	100±4.9B	102±3.7B	101±5.7B	101±3.0B	97.4±3.9B	96.8±1.7B	99.6±3.6B	102±3.9B	101±5.9B	98.3±5.9C	101±1.6B
9	153±7.1A	139±5.4BC	145±7.2AB	147±8.0AB	133±6.2BC	142±9.3B	131±2.9CD	146±7.7AB	140±7.0BC	140±11AB	129±7.0D	145±2.3AB
12	190±11ABC	[illegible]±8.7CD	196±15ABC	194±9.6A-D	194±4.4A-D	170±8.7D	177±7.0D	189±8.3BCD	184±17BCD	207±17A	196±17BCD	201±6.9AB
15	245±12BC	[illegible]±9.0EF	243±22CDE	244±5.8CD	230±3.8C-F	220±11F	225±7.3DEF	230±6.7C-F	244±22CD	272±23A	240±14CDE	264±11AB
18	304±14CDE	275±[illegible]F	302±31CDE	296±9.8DEF	296±9.0DEF	279±12EF	290±4.3EF	281±12EF	326±75ABC	350±28A	319±21BCD	349±12AB
21	354±20BDE	331±17DE	368±43C	359±14CD	357±15.3CDE	376±13C	358±8.4CDE	330±14CDE	399±33B	436±34A	404±35AB	432±17A
24	435±23DEF	395±20F	443±54CDE	427±11DEF	435±11.0DEF	406±14EF	450±14CD	425±19DEF	479±44BC	520±29A	490±30AB	525±29A
25	459±20DE	424±25EF	471±80CD	449±14DE	460±11.1DE	405±16F	456±34DE	420±19EF	509±52BC	560±41AB	575±36AB	557±71A
28	481±22DE	446±25EF	490±60CD	465±17DEF	460±11.1DE	430±18F	482±21DE	439±27EF	537±44BC	574±44AB	556±41AB	586±43A

[a]Values (means ± standards) for weight gain followed by the same letter are not significantly different at P≤0.05. Initial number of 24 chicks per treatment was reduced to 20 chicks at 7 days.

[b]Pair fed to the feed intake of the corresponding aflatoxin group.

[c]Percentage of the NRC dietary requirement (1977) of lysine or arginine (basal ration provides 94% and 102%, respectively).

App. 14a. Major and interactive effects produced by graded levels of dietary lysine and arginine on weight gain of chicks with aflatoxicosis.

Day	Weight gain (g/chick)[a]							ANOVA (F[Pr > F])[e]		
	Effect of feeding			Effect of administration[d]						
	Aflatoxin[b]	Restricted[c]	Ad libitum	94Arg+102Lys	122Arg+102Lys	122Arg+122Lys	122Arg+146Lys	Feed	Admin	Feed x Admin
0	-A	-A	-A	-A	-A	-A	-A	1.38(0.27)	0.14(0.94)	0.99(0.45)
3	-A	-A	-A	-A	-A	-A	-A	1.48(0.24)	1.71(0.18)	0.96(0.47)
6	103A	99.1B	98.8B	104A	100B	96C	101AB	7.25(0.0023)	7.37(0.0006)	2.33(0.038)
9	146A	140B	140B	144A	142A	135B	146A	4.45(0.019)	6.18(0.0017)	2.73(0.028)
12	193A	184B	194A	-A	-A	-A	-A	3.92(0.029)	1.40(0.26)	3.51(0.0077)
15	239B	228C	256A	-A	-A	-A	-A	17.1(0.0001)	0.95(0.43)	3.31(0.011)
18	294B	287B	335A	-A	-A	-A	-A	32.0(0.0001)	0.38(0.77)	2.56(0.036)
21	352B	345B	418A	-A	-A	-A	-A	44.4(0.0001)	0.63(0.61)	2.51(0.040)
24	24.5B	429B	505A	-A	-A	-A	-A	38.7(0.0001)	1.47(0.24)	2.15(0.071)
25	451B	435B	535A	-A	-A	-A	-A	46.1(0.0001)	1.34(0.28)	2.55(0.037)
26	472B	457B	561A	-A	-A	-A	-A	38.6(0.0001)	1.17(0.34)	2.16(0.070)

[a]Means for the gain in weight within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin 2.5μg per g of diet.

[c]Pair-fed to the feed intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of lysine or arginine (basal ration provided 102% and 98% respectively).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 15. Effect of graded levels of lysine and arginine of feed conversion of chicks with aflatoxicosis.[a]

Day	Aflatoxin (2.5μg/g of diet)				Restricted Feeding[b]				Ad Libitum Controls			
	94 Arg[c]	122 Arg			94 Arg	122 Arg			94 Arg	122 Arg		
	102 Lys[c]	102 Lys	122 Lys	146 Lys	102 Lys	102 Lys	122 Lys	146 Lys	102 Lys	102 Lys	122 Lys	146 Lys
3	1.26±0.1D	1.31±0.1CD	1.25±0.1D	1.34±0.1BCD	1.35±0.2BCD	1.27±0.2D	1.50±0.1AB	1.30±0.0CD	1.44±0.1ABC	1.32±0.1CD	1.60±0.1A	1.31±0.1CD
6	1.53±0.1G	1.73±0.1C-F	1.59±0.1FG	1.63±0.1EFG	1.67±0.1D-G	1.81±0.2BCD	1.90±0.1B	1.67±0.1D-G	1.83±0.2BC	1.78±0.1B-E	2.23±0.1A	1.68±0.0C-G
9	1.63±0.1D	1.82±0.1CD	1.68±0.1D	1.74±0.1CD	1.81±0.1CD	1.72±0.2CD	2.05±0.3AB	1.69±0.0D	1.91±0.2BC	1.83±0.1CD	2.25±0.1A	1.78±0.1CD
12	1.74±0.1CD	1.87±0.1BCD	1.72±0.1D	1.79±0.1CD	1.76±0.1CD	1.90±0.2BC	2.04±0.1AB	1.82±0.1CD	2.08±0.3A	1.83±0.1CD	2.13±0.1A	1.83±0.0CD
15	1.88±0.1CD	2.01±0.1BC	1.83±0.1D	1.85±0.1D	1.89±0.0CD	1.97±0.1BCD	2.05±0.1AB	1.97±0.1BCD	2.09±0.2AB	1.87±0.1CD	2.18±0.1A	1.86±0.0CD
18	2.00±0.0BCD	2.14±0.1AB	1.95±0.1CD	1.97±0.1CD	1.97±0.1CD	[illegible]±0.1BCD	2.02±0.1BCD	2.03±0.1A-D	2.08±0.2ABC	1.95±0.1CD	2.18±0.1A	1.91±0.1D
21	2.18±0.1A	2.12±0.0AB	2.00±0.1CDE	2.01±0.1B-E	2.05±0.1A-E	2.11±0.1A-D	2.01±0.1B-E	2.08±0.1A-E	2.15±0.2ABC	1.97±0.1DE	2.13±0.1A-D	1.93±0.1E
24	2.22±0.1AB	2.28±0.1A	2.06±0.1C	2.08±0.1BC	2.13±0.1ABC	2.09±0.1BC	1.97±0.1C	2.02±0.1C	2.27±0.2A	2.06±0.1C	2.12±0.1ABC	1.98±0.1C
25	2.24±0.1ABC	2.23±0.1ABC	2.06±0.1CD	2.10±0.1BCD	2.14±0.1A-D	2.26±0.1AB	2.10±0.1BCD	2.19±0.1ABC	2.28±0.2A	2.09±0.1BCD	2.13±0.1A-D	1.99±0.1D
26	2.26±0.1AB	2.24±0.1AB	2.06±0.1BC	2.12±0.1ABC	2.19±0.1ABC	2.26±0.1AB	2.11±0.1ABC	2.22±0.1AB	2.32±0.3A	2.10±0.1BC	2.13±0.1ABC	1.99±0.1C

[a]Values (means ± standard deviations) for feed conversion followed by the same letter are not significantly different at $P \leq 0.05$. Initial number of 24 chicks per treatment was reduced to 20 chicks at 7 days.

[b]Pair fed to the feed intake of the corresponding aflatoxin group.

[c]Percentage of the NRC dietary requirement (1977) of lysine or arginine (basal ration provides 94% and 102%, respectively).

App. 15a. Major and interactive effects produced by graded levels of dietary lysine and arginine on feed conversion of chicks with aflatoxicosis.

Day	Feed conversion (g/chick)[a]							ANOVA (F[Pr >F])[e]		
	Effect of feeding			Effect of administration[d]						
	Aflatoxin[b]	Restricted[c]	Ad libitum	94Arg+102Lys	122Arg+102Lys	122Arg+122Lys	122Arg+146Lys	Feed	Admin	Feed x Admin
3	1.29B	1.35AB	1.42A	1.35B	1.30B	1.45A	1.32B	5.39(0.0090)	4.38(0.0099)	2.96(0.019)
6	1.62C	1.76B	1.88A	1.68C	1.77B	1.91A	1.66C	19.1(0.0001)	10.8(0.0001)	5.54(0.0004)
9	1.72B	1.82B	1.94A	1.78B	1.79B	1.99A	1.74B	9.44(0.0005)	7.21(0.0007)	3.70(0.0050)
12	1.78C	1.88B	1.97A	1.86AB	1.87AB	1.96A	1.81B	9.54(0.0005)	3.21(0.034)	4.70(0.0013)
15	1.89B	1.97A	2.00A	1.95AB	1.95AB	2.02A	1.89B	4.92(0.013)	3.11(0.038)	5.25(0.0006)
18	-A	-A	-A	-A	-A	-A	-A	0.37(0.69)	1.45(0.24)	4.46(0.0018)
21	-A	-A	-A	2.13A	2.08AB	2.05AB	2.00B	0.80(0.46)	3.40(0.028)	3.27(0.011)
24	2.16A	2.05B	2.11AB	2.21A	2.14A	2.05B	2.02B	4.09(0.025)	7.47(0.0005)	1.74(0.14)
25	-A	-A	-A	2.22A	2.19A	2.10B	2.09B	0.85(0.43)	4.34(0.010)	2.53(0.038)
26	-A	-A	-A	2.26A	2.20AB	2.11B	2.10B	0.89(0.42)	3.98(0.015)	1.90(0.11)

[a]Means of feed conversion rates (feed intake/weight gain) within a major effect followed by the same letter are not significantly different at P≤0.05.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the feed intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of lysine or arginine (basal ration provided 102% and 94% respectively).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 16. Effect of administrations of nutrients and aflatoxicosis on mortality and also on hepatic weight, moisture and lipid.

Hepatic Parameter	Hepatic weight, moisture or lipid content[a]											
	Aflatoxin (2.5 µg/g of diet)				Restricted Feeding[b]				Ad libitum			
	A	B	C	D[c]	A	B	C	D	A	B	C	D
Choline[c]												
Weight (g)	22.0±2.1A	20.4±1.2AB	20.8±3.7AB	-	17.0±1.8CD	14.2±2.4D	17.0±1.4CD	-	18.5±1.3BC	16.9±1.2CD	19.3±1.0ABC	-
Moisture (%)	73.0±1.2A	74.6±2.4A	72.3±1.5A	-	74.4±0.9A	74.1±1.9A	74.2±1.2A	-	74.3±1.1A	74.3±0.5A	74.8±0.5A	-
Lipid (% DWB)	31.4±4.2A	32.1±4.2A	27.5±3.6B	-	12.9±2.0C	14.3±2.8C	12.5±1.1C	-	14.0±2.1C	14.1±1.4C	14.9±1.1C	-
Mortality 7-days	2	1	2	-	0	1	0	-	2	4	1	-
24-days	2	5	3	-	0	1	2	-	3	5	1	-
Folate[c]												
Weight (g)	18.6±1.7AB	19.6±0.5A	16.6±2.0BC	-	16.0±2.7BC	17.5±1.6ABC	18.2±1.7ABC	-	18.0±0.8ABC	15.5±0.9C	17.2±1.4ABC	-
Moisture (%)	69.9±0.8E	74.3±1.8AB	73.9±1.4ABC	-	72.3±0.3D	72.5±1.3CD	73.0±0.4BCD	-	73.8±0.7ABC	74.6±0.5A	73.8±0.9ABC	-
Lipid(% DWB)	27.4±2.1A	28.9±2.8A	28.2±2.4A	-	15.9±3.2BC	17.5±1.1B	15.3±1.2BC	-	14.1±1.2C	14.2±0.8C	13.9±1.4C	-
Mortality 7-days	0	0	1	-	0	0	0	-	0	0	0	-
24-days	1	1	1	-	0	1	0		0	0	0	-
Threonine[c]												
Weight (g)	20.0±0.7A	19.9±1.0A	20.2±0.6A	-	16.9±0.4CD	17.5±0.6BC	16.1±0.7D	-	16.7±0.9CD	16.1±0.9D	18.0±1.1B	-
Moisture (%)	76.0±0.4A	76.1±1.0A	75.6±1.1AB	-	73.7±0.2D	72.6±0.3E	73.7±0.9D	-	74.8±0.3BC	74.4±0.4CD	74.3±0.5CD	-
Lipid (% DWB)	21.9±3.4B	18.4±1.3C	25.3±2.3A	-	14.9±0.7DE	16.6±2.5CD	13.9±0.9E	-	13.9±1.0E	15.4±1.9DE	13.7±1.2E	-
Mortality 7-days	0	0	0	-	1	1	1	-	1	2	0	-
24-days	1	1	1	-	2	1	2	-	2	3	0	-
Lysine[c]												
Weight (g)	16.8±1.9ABC	18.3±1.4A	17.4±1.6AB		13.0±0.5D	12.6±1.0D	12.9±0.6D	-	15.4±1.4C	16.0±1.4BC	15.6±1.0C	-
Moisture (%)	75.1±0.8AB	75.1±0.6AB	75.8±0.4A		75.2±0.2AB	74.7±0.6AB	75.0±0.3AB	-	75.1±0.8AB	74.9±0.5AB	74.7±0.3B	-
Lipid (% DWB)	27.4±2.0A	26.6±2.4A	28.4±3.2A		14.1±0.5C	15.5±1.9BC	15.2±0.8BC	-	16.7±1.5B	15.2±1.4BC	15.2±0.9BC	-
Mortality 7-days	1	1	1		0	0	2		0	0	1	-
24-days	1	2	1	-	0	0	3		2	0	1	-
Lysine + arginine[c]	1	2	1		0	0	3		2	0		
Moisture (%)	73.3±1.5	70.0±3.5	74.0±5.2	69.0±4.0	73.3±3.0	74.8±3.1	77.5±1.3	72.5±2.4	74.5±1.0	72.5±3.1	74.3±2.6	72.3±0.96
Lipid (% DWB)[d]	30.4±5.4BC	35.3±8.1A	26.6±4.3C	31.1±3.6AB	13.9±1.0D	13.7±0.7D	14.3±0.2D	14.0±0.7D	15.1±0.9D	14.4±1.4D	14.3±0.3D	14.3±1.7D
Mortality 7-days	2	0	0	0	0	0	0	0	0	0	0	0
24-days	2	0	0	1	0	1	0	2	0	0	0	0

[a] Values (means ± SEM) for a parameter followed by the same letter are not significantly different at P ≤0.05.
[b] Pair fed to the feed intake of the corresponding aflatoxin group.
[c] A,B,C or D correspond to the three or four nutritional administrations provided in the five feeding trials (choline: no supplement, interperitoneal or dietaty supplements to achieve 175% of the NRC (1977) requirement, folate: 244, 344 or 644% NRC, threonine: 128, 155 or 179% NRC; lysine: 102, 122 or 146% NRC; lysine + arginine: 102/94, 102/122, 122/122 or 146/122 % NRC). Basal ratios provided the lowest % NRC values, but 106% of the NRC in the choline study.
[d] DWB = dry weight basis.

App. 16a. Major and interactive effects produced by administrations of nutrients and aflatoxin on hepatic weight, moisture and lipid.

	Hepatic moisture, weight or lipid[a]							ANOVA (F[Pr>F][e])		
	Effect of feeding			Effect of Administration[d]						
Hepatic Parameter	Aflatoxin[b]	Restricted[c]	Ad libitum	A	B	C	D	Feed	Admin	FeedxAdmin
Choline[c]	21.0A	16.3C	18.2C	A	A	A		18.3(0.0001)	3.00(0.066)	0.41(0.80)
Weight (g)	A	A	A	A	A	A		1.96(0.16)	0.36(0.70)	1.01(0.42)
Moisture (%)	30.3A	13.2B	14.4B	A	A	A		144(0.0001)	1.54(0.23)	1.13(0.36)
Lipid (% DWB)										
Folate[c]										
Weight (g)	A	A	A	A	A	A		2.26(0.12)	0.07(0.93)	4.13(0.0097)
Moisture (%)	72.7B	72.6B	74.0A	72.0B	73.8A	73.6A		7.58(0.0024)	11.0(0.0003)	6.70(0.0007)
Lipid (% DWB)	28.2A	16.2B	14.1C	A	A	A		165(0.0001)	1.07(0.36)	0.35(0.84)
Threonine[c]										
Weight (g)	20.0A	16.8B	16.9B	A	A	A		65.8(0.0001)	0.43(0.65)	4.50(0.0064)
Moisture (%)	75.9A	73.3C	74.5B	A	A	A		48.5(0.0001)	1.61(0.22)	1.77(0.16)
Lipid (% DWB)	21.8A	15.1B	14.3B	A	A	A		57.3(0.0001)	0.66(0.53)	7.89(0.0002)
Lysine[c]										
Weight (g)	17.5A	12.8C	15.7B	A	A	A		42.4(0.0001)	0.51(0.61)	0.62(0.65)
Moisture (%)	A	A	A	A	A	A		2.43(0.11)	0.84(0.44)	1.87(0.15)
Lipid (% DWB)	27.5A	14.9B	15.7B	A	A	A		181(0.0001)	0.28(0.76)	1.15(0.35)
Lysine + argine[c]										
Lipid (% DWB)	30.5A	14.1B	15.0B	17.8B	17.7B	21.9A	18.7B	272(0.0001)	6.70(0.033)	2.76(0.14)

[a]Means of a parameter within a major effect followed by the same letter are not significantly different at $p \leq 0.05$.
[b]Pair fed to the feed intake of the corresponding aflatoxin group.
[c]A,B,C or D correspond to the three or four nutritional administrations provided in the five feeding trials (choline: no supplement, interperitoneal or dietary supplements to achieve 175% of the NRC (1977) requirement, folate: 244, 344 or 644% NRC, threonine: 128, 155 or 179% NRC; lysine: 102, 122 or 146% NRC; lysine + arginine: 102/94, 102/122, 122/122 or 146/122% NRC). Basal ratios provided the lowest % NRC values, but 106% of the NRC in the choline study.
[d]DWB = dry weight basis.
[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 17. Effect of dietary or interperitoneal administration (IP) of choline on various components in plasma of chicks with aflatoxicosis.

	Plasma parameter[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Ad libitum Control		
Parameter (units)	0[c]	IP[d]	Dietary[d]	0	IP	Dietary	0	IP	Dietary
Calcium	4.8±0.48C	5.3±0.68C	4.7±0.38C	7.0±0.62AB	6.7±0.39B	7.3±0.19A	7.1±0.35AB	7.2±0.26AB	7.2±0.69A
Phosphorus, inorg. (mg %)	4.8±0.65C	5.4±0.21B	5.3±0.37BC	5.8±0.37B	5.5±0.28B	5.7±0.34B	6.6±0.33A	6.4±0.13A	6.5±0.29A
Total iron (μg %)	73±8.5DE	84±6.7D	64±5.5E	105±8.5BC	117±4.1A	99±5.8C	116±11AB	112±13AB	121±9.8A
TIBC[e] (μg%)	113±12B	166±10A	97±0.71B	166±18A	179±7.1A	159±5.7A	150±17A	159±12A	163±3.5A
Saturated transferrin (%)	70±0.0BC	55±23D	71±3.5BC	72±5.7BC	67±0.71C	72±8.5BC	83±2.8A	75±4.2ABC	80±2.1AB
Glucose (mg %)	249±3.6C	247±1.2C	250±3.8C	252±5.4C	252±6.7C	249±2.3C	261±4.2B	267±2.1A	260±4.2B
Cholesterol (mg %)	54±5.3DE	61±0.58D	48±7.0E	125±5.1B	127±7.3B	139±9.5A	111±15C	114±4.5C	115±3.2C
Triglycerides (mg %)	85.3±10A	81.8±4.6A	71.1±7.8B	54.1±6.1D	61.2±4.5CD	57.4±3.8D	61.8±1.1CD	68.5±5.1BC	63.3±9BCD
Glycerol (μg%)	496±116A	440±123A	386±46AB	256±84BC	265±169BC	174±78C	130±86C	255±42BC	177±42C
Total protein (g %)	1.3±0.96D	2.0±0.15C	1.3±0.17D	3.0±0.17AB	2.8±0.10B	3.0±0.10AB	3.0±0.15A	3.0±0.13AB	3.1±0.10A
Albumin (g %)	0.55±0.58D	0.73±0.05C	0.50±0.08D	1.4±0.06AB	1.4±0.06AB	1.3±0.12B	1.4±0.06AB	1.4±0.05AB	1.4±0.0A
Globulin (g %)	0.73±0.05D	1.3±0.14C	0.75±0.13D	1.6±0.16AB	1.5±0.13B	1.7±0.05A	1.7±0.10A	1.6±0.10AB	1.7±0.10A
Albumin/globulin ratio	0.76±0.07BC	.56±0.07D	0.68±0.14CD	0.85±0.10AB	0.92±0.11A	0.77±0.09BC	0.81±0.02AB	0.88±0.05AB	0.84±0.05A
Blood urea nitrogen (mg%)	1.5±0.58BC	2.8±0.5A	2.3±0.5AB	1.5±0.58BC	1.5±0.58BC	1.3±0.50C	2.0±0.0ABC	2.0±0.82ABC	1.8±0.50B
Uric acid (mg %)	5.6±0.08B	5.6±0.96B	5.3±0.26B	4.2±0.70C	3.8±1.1C	3.8±0.69C	7.5±0.08A	7.3±0.04A	7.5±0.17A
Total bilirubin (mg %)	0.20±0.0A	0.20±0.0A	0.20±0.0A	0.20±0.0A	0.20±0.0A	0.18±0.05A	0.20±0.0A	0.20±0.0A	0.18±0.05A
Alkaline phosphatase (U/L)	3500±0.0A	3475±248A	3485±233A	3500±0.0A	3500±0.0A	3500±0.0A	3540±57A	3285±304A	3500±0.0A
LDH[e] (U/L)	881±45A	855±17A	758±44A	508±7.5D	610±69C	443±17E	426±80E	437±61E	446±18E
AST[e] (U/L)	168±5.1F	194±6.7CDE	151±17G	206±7.7BC	229±12A	214±6.4B	187±10E	191±17BE	203±8.7BC
ALT[e] (U/L)	ND[f]	ND	ND	ND	ND	ND	ND	ND	ND
SAL[e] (pmol/h/ul)	4.8±0.11B	6.4±0.91A	4.5±0.13B	6.2±0.33A	6.5±0.55A	6.5±0.36A	6.4±0.30A	6.6±0.36A	6.0±0.17A
GAL[e] (pmol/h/ul)	48±5.7AB	56±9.3AB	59±11AB	58±3.2AB	62±6.1A	64±6.1A	43±20B	63±6.4A	61±8.6A
Folate (ng/ml)	21.8±6.1A	19.3±2.6A	19.3±2.8A	17.6±5.2A	17.7±3.5A	22.9±4.9A	18.8±3.1A	21.0±4.9A	22.9±3.0A
Choline (ng/ml)	26.0±2.7E	27.8±7.1DE	25.5±4.4E	36.8±11CDE	55.5±9.1AB	52.0±6.7AB	46.5±13BC	62.3±12A	39.5±5.7CD

[a] Values (SEM) for a clinical parameter followed by the same letter are not significantly different at $P \leq 0.05$. Creatinine was not detected (0.10 mg%).

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] No supplemental choline.

[d] Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provides 106%).

[e] TIBC = total iron binding capacity, LDH = lactate dehydrogenase, AST = asparate aminotransferase, ALT = alanine aminotransferase, SAL = sialyltransferase, GAL = galactosyltransferase.

[f] ND = not detected at less than 0.1 U/L.

App. 17a. Major and interactive effects produced by dietary or intraperitoneal (IP) of choline on various components in plasma of chicks with aflatoxicosis.

Parameter (units)	Plasma parameter[a]								
	Effect of feeding			Effect of administration			ANOVA (F [Pr > F])[f]		
	Aflatoxin[g]	Restricted[b]	Ad libitum	0[c]	IP[e]	Dietary[e]	Feed	Admin	Feed x Admin
Calcium (mg%)	4.88B	6.99A	7.17A	A	A	A	84.7(0.0001)	0.13(0.88)	1.77 (0.17)
Phosphorus, inorg. (mg%)	5.18C	5.67B	6.45A	A	A	A	39.1(0.0001)	0.33(0.73)	1.84(0.15)
Total iron (µg%)	73.6C	107B	116A	97.9AB	104A	94.3B	75.1(0.0001)	3.93(0.032)	3.38(0.023)
TIBC[d] (µg%)	125B	168A	157A	143B	168A	139B	17.7(0.0008)	8.57(0.0087)	4.40(0.031)
Saturated transferin (%)	65.0B	70.2B	79.2A	75.0A	65.3B	74.0A	18.8(0.0006)	10.3(0.0047)	1.47(0.29)
Folic acid (ng/ml)	A	A	A	A	A	A	0.40(0.67)	1.21(0.31)	1.12(0.37)
Glucose (mg%)	249B	251B	262A	A	A	A	38.1(0.0001)	1.09(0.35)	2.05(0.12)
Cholesterol (mg%)	53.9C	130A	113B	A	A	A	353(0.0001)	1.20(0.32)	3.19(0.029)
Triglycerides (mg%)	794A	592C	645AB	687AB	705A	639B	31.7(0.0001)	3.40(0.048)	1.75(0.17)
Glycerol (µg%)	441A	232B	187B	A	A	A	23.8(0.0001)	1.87(0.17)	1.15(0.36)
Total protein (g%)	1.52C	2.92B	30.2A	2.42B	2.60A	2.43B	480(0.0001)	7.01(0.0035)	19.7(0.0001)
Albumin (g%)	0.59B	1.33A	1.38A	1.08B	1.15A	1.07B	547(0.0001)	5.48(0.010)	4.50(0.0065)
Globulin (g%)	0.93B	1.58A	1.64A	1.33B	1.45A	1.37AB	153(0.0001)	3.49(0.045)	17.5(0.0001)
Albumin/globulin ratio	0.67B	0.85A	0.84A	A	A	A	18.4(0.0001)	0.73(0.49)	4.29(0.0082)
Blood urea nitrogen (mg%)	2.17A	1.42B	1.92A	A	A	A	5.91(0.0075)	1.97(0.16)	1.97(0.13)
Uric acid (mg%)	5.50B	3.91C	7.43A	A	A	A	140(0.0001)	0.56(0.58)	0.34(0.85)
Total bulirubin (mg%)	A	A	A	A	A	A	0.50(0.01)	2.00(0.16)	0.50(0.74)
Alkaline phosphatase (U/L)	A	A	A	A	A	A	0.24(0.79)	0.62(0.56)	0.50(0.74)
LDH[d] (U/L)	831A	521B	436C	634A	605A	549B	348(0.0001)	15.1(0.0001)	7.72(0.0003)
AST[d] (U/L)	171C	216A	194B	187B	205A	189B	52.3(0.0001)	9.37(0.0008)	689(0.0005)[a]
Choline (ng/ml)	26.4B	48.1A	49.4A	36.4B	48.5A	39.0B	24.7(0.0001)	5.99(0.007)	2.84(0.04)

[a]Means for a clinical parameter within a major effect followed by the same letter are not significantly different at P≤0.05.
[b]Pair fed to the feed intake of the corresponding aflatoxin group.
[c]No supplemental choline
[d]TIBC = total iron binding capacity, LDH = lactate dehydrogenase, AST = aspartate aminotransferase.
[e]Administration to achieve 175% of NRC requirement (1977) of choline (basal ration provided 106%).
[f]Admin = major effect of administraion. Feed = major effect of feeding. Feed x Admin = test for interaction.
[g]Dietary aflatoxin = 2.5 µg per of diet.

App. 18. Effect of graded levels of dietary folic acid on various components in plasma of chicks with aflatoxicosis.

	Plasma parameter[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Ad libitum Control		
Parameter (units)	244[c]	344[c]	644[c]	244	344	644	244	344	644
Calcium	7.4±0.66C	7.8±0.36C	7.5±0.51C	9.3±0.26A	9.1±0.31AB	8.8±0.24B	9.3±0.08AB	9.0±0.30AB	9.4±0.17A
Phosphorus, inorg. (mg %)	6.0±0.14EF	5.8±0.18F	6.1±0.57DEF	7.4±0.33A	7.4±0.08A	7.1±0.31AB	6.3±0.24CDE	6.6±0.21CD	6.8±0.60B
Total iron (μg %)	78±7.5C	88±2.5C	79±9.4C	112±5.4A	109±6.2AB	108±3.0AB	100±12B	108±5.4AB	114±6.9A
TIBC[d] (μg%)	111±17C	122±26BC	125±17ABC	129±12ABC	122±8.2BC	130±11AB	139±13AB	124±8.1ABC	150±17A
Saturated transferrin (%)	69±11C	73±13BC	69±14C	86±5.6AB	89±3.6A	85±2.6AB	77±9.7ABC	87±2.9AB	76±7.6AB
Glucose (mg %)	276±10C	283±4.8ABC	286±12ABC	289±4.1AB	295±3.9A	294±6.3A	283±6.6ABC	279±8.5BC	284±8.2AB
Cholesterol (mg %)	47±7.0C	45±6.8C	41±6.4C	113±6.9A	116±5.0A	114±5.6A	87±13B	85±6.3B	92±4.6B
Triglycerides (mg %)	69.4±10A	73.6±5.3A	75.1±15A	72.4±3.8A	76.3±9.2A	76.3±6.7A	69.2±4.9A	68.7±3.5A	70.8±6.7A
Glycerol (μg%)	416±105A	491±243A	429±60A	345±115A	236±103A	438±205A	237±45A	343±124A	305±135A
Total protein (g %)	1.7±0.24C	1.7±0.26C	1.5±0.15C	3.0±0.22A	2.8±0.13B	2.7±0.12B	2.8±[illegible]0AB	2.6±0.13B	2.8±0.00B
Albumin (g %)	0.65±0.17C	0.68±0.10C	0.55±0.06C	1.5±0.15A	1.4±0.06AB	1.3±0.15B	1.4±0.06AB	1.2±0.05B	1.4±0.06A
Globulin (g %)	1.0±0.08B	1.0±0.18B	0.93±0.10B	1.6±0.13A	1.4±0.10A	1.4±0.05A	1.5±0.05A	1.4±0.08A	1.5±0.06A
Albumin/globulin ratio	0.65±0.15B	0.68±0.07B	0.60±0.03B	0.96±0.10A	0.95±0.06A	0.97±0.14A	0.92±0.03A	0.88±0.03A	0.93±0.08A
Blood urea nitrogen (mg%)	2.5±0.58A	2.0±0.00AB	2.0±0.00AB	2.3±0.50AB	1.3±0.50B	2.0±0.82AB	1.8±0.50AB	1.5±1.0AB	1.8±0.50A
Uric acid (mg %)	5.0±0.49DE	5.1±0.46CD	4.3±0.41E	6.7±0.68A	6.0±0.70AB	6.4±0.72AB	5.8±0.34BC	5.8±0.46BC	6.2±0.52A
Total bilirubin (mg %)	0.33±0.05A	0.28±0.05AB	0.25±0.06AB	0.23±0.05AB	0.25±0.06AB	0.28±0.05AB	0.25±0.06AB	0.20±0.00B	0.28±0.10A
Creatinine (mg %)	ND[e]	ND	ND	0.60±0.69	0.10±0.20	0.18±0.35	ND	0.33±0.22	ND
Alkaline phosphatase (U/L)	3680±733BC	3995±677ABC	3281±693C	3820±203ABC	4763±511AB	4135±688ABC	4805±1125AB	4645±756AB	5135±1128A
LDH[d] (U/L)	847±91AB	891±34A	809±46B	465±57CD	487±72CD	521±31C	444±25D	429±51D	492±21CD
AST[d] (U/L)	181±8.5C	212±19A	192±15BC	224±17A	208±14AB	214±3.9A	207±6.7AB	209±6.5AB	220±10A
ALT[d] (U/L)	1.8±1.0BCD	4.0±0.82A	2.5±1.3B	2.0±1.2BC	1.5±1.0BCD	1.8±1.0BCD	0.75±0.50CD	0.50±1.0D	2.0±0.08C
SAL[d] (pmol/h/ul)	5.1±0.72AB	4.6±0.33ABC	5.2±0.51A	3.7±0.34DE	4.2±0.71CDE	3.7±0.39E	4.8±0.40ABC	4.3±0.59B-E	4.5±0.48A
CAL[d] (pmol/h/ul)	67.1±5.6A	55.6±2.7BC	60.9±7.6AB	53.8±3.6C	56.6±4.2BC	56.2±2.9BC	58.1±2.4BC	54.2±3.8BC	56.2±3.6BC
Folic acid- plasma(ng/ml)	6.16±2.2A	15.8±6.8B	15.0±2.1B	5.30±1.7A	6.40±2.5A	8.08±1.2A	8.01±1.6A	16.3±2.9B	17.3±5.7B
Folic acid-bile(ng/ml)	171±155BC	359±206A	346±238AB	45.4±3.0C	52.6±16C	56.0±23C	85.9±52C	194±60ABC	301±73AB

[a] Values (means ± SEM) for a clinical parameter followed by the same letter are not significantly different at $P \leq 0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of folate (basal ration provides 244%).

[d] TIBC = total iron binding capacity, LDH = lactate dehydrogenase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, SAL = sialyltransferase, GAL = galactosyltransferase.

[e] ND = not detected at less than 0.01 mg %.

App. 18a Major and interactive effects produced by graded levels of dietary folic acid on various components in plasma of chicks with aflatoxicosis.

Parameter (units)	Plasma parameter[a]								
	Effect of feeding			Effect of administration[e]			Anova [F (Pr>F)][f]		
	Aflatoxin[b]	Restricted[c]	Ad libitum	128	155	179	Feed	Admin	Feed x Admin
Calcium (mg%)	7.57B	9.07A	9.23A	-A	-A	-A	77.9(0.0001)	0.10(0.90)	2.63(0.056)
Phosphorus, inorg. (mg%)	5.98C	7.30A	6.55B	-A	-A	-A	46.0(0.0001)	0.28(0.76)	1.52(0.22)
Total iron (μg%)	81.6B	109A	107A	-A	-A	-A	59.1(0.0001)	1.49(0.24)	2.55(0.062)
TIBC[d] (μg%)	119B	127AB	138A	-A	-A	-A	4.40(0.022)	2.00(0.15)	1.08(0.39)
Saturated transferin (%)	70.1C	79.8B	86.8A	-A	-A	-A	11.0(0.0003)	1.71(0.20)	0260(0.90)
Glucose (mg%)	282B	293A	282B	-A	-A	-A	8.18(0.0017)	1.56(0.23)	0.81(0.53)
Cholesterol (mg%)	44.3C	114.0A	88.0B	-A	-A	-A	283(0.0001)	0.01(0.99)	1.02(0.42)
Triglycerides (mg%)	A	A	A	A	A	A	1.42(0.26)	0.67(0.52)	0.14(0.97)
Glycerol (μg%)	445A	339AB	295B	A	A	A	3.67(0.04)	0.52(0.60)	1.25(0.31)
Total protein (g%)	1.60B	2.83A	2.75A	2.50A	2.36AB	2.33B	204(0.0001)	3.71(0.038)	1.92(0.14)
Albumin (g%)	0.63B	1.38A	1.31A	-A	-A	-A	189(0.0001)	2.28(0.12)	1.75(0.17)
Globulin (g%)	0.98B	1.45A	1.44A	1.34A	1.28AB	1.25B	88.7(0.0001)	2.69(0.086)	0.94(0.45)
Albumin/globulin ratio	0.64B	0.96A	0.91A	-A	-A	-A	43.9(0.0001)	0.02(0.98)	0.71(0.60)
Blood urea nitrogen (mg%)	-A	-A	-A	2.17A	1.92AB	1.58B	2.33(0.12)	3.08(0.062)	0.71(0.59)
Uric acid (mg%)	4.80C	6.33A	5.92B	-A	-A	-A	25.4(0.0001)	0.41(0.67)	2.07(0.11)
Total bilirubin (mg%)	-A	-A	-A	-A	-A	-A	1.80(0.19)	0.77(0.47)	1.80(0.16)
Alkaline phosphatase (U/L)	3652B	4239AB	4862A	-A	-A	-A	7.37(0.0028)	0.74(0.49)	1.04(0.40)
LDH[d] (U/L)	849A	491B	455B	-A	-A	-A	209(0.0001)	0.58(0.57)	2.34(0.08)
AST[d] (U/L)	212A	215A	195B	-A	-A	-A	9.49(0.0008)	0.72(0.49)	4.38(0.0074)
ALT[d] (U/L)	2.75A	1.75B	1.08B	-A	-A	-A	9.81(0.0006)	1.39(0.27)	4.00(0.011)
Folic acid, plasma (ng/ml)	12.3A	6.59B	13.8A	6.49B	12.8A	13.4A	14.5(0.0001)	14.6(0.0001)	1.96(0.13)
Folic acid, bile (ng/ml)	292A	51.4C	194B	101B	202AB	234A	11.7(0.0002)	3.86(0.034)	1.06(0.39)

[a]Means for a clinical parameter within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]TIBC = total iron binding capacity. LDH = lactate dehydrogenase. AST = aspartate aminotransferase. ALT = alanine aminotransferase.

[e]Percentage of the NRC dietary requirement (1977) of lysine (basal ration provided 244%).

[f]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 19. Effect of graded levels of dietary threonine on the plasma biochemistry of chicks with aflatoxicosis.

Parameter (units)	Plasma parameter[a]								
	Aflatoxin (2.5 µg/g of diet)			Restricted Feeding[b]			Control		
	128[c]	155[c]	179[c]	128	155	179	128	155	179
Calcium	4.2±0.26C	4.2±0.33C	4.4±0.37C	6.7±0.3AB	6.9±0.29A	6.5±0.15AB	6.6±0.42AB	6.4±0.28B	6.4±0.[illegible]
Phosphorus, inorg. (mg %)	4.1±0.1E	4.2±0.19E	[illegible]±0.24E	4.9±0.14AB	4.6±0.30D	4.8±0.29BCD	4.6±0.19CD	5.1±0.13A	4.8±0.13A
Total iron (µg %)	60.8±14C	51.8±16C	62.3±.1C	130±16A	111±19AB	103±23B	108±14AB	110±11AB	110±23AB
TIBC[d]	89.9±9.8E	119±5.0D	126±12D	171±21ABC	184±13A	169±14ABC	164±10BC	155±9.0[illegible]	175±19AB
Saturated transferin (%)	51.8±6.2BCD	43±16D	46.3±11CD	76±2.8A	60.8±14A-C	62.8±17ABC	66.5±11AB	70.5±9.7A	62.5±12ABC
Glucose (mg %)	262±4.3C	258±5.4C	259±7.8C	283±6.6AB	281±7.0AB	286±4.4A	263±1.5C	274±5.6B	275±8.4B
Cholesterol (mg %)	50.5±7.0D	42.5±2.5D	47.5±6.9D	123±6.6B	149±15A	125±3.9B	109±4.9C	127±4.1B	106±1.8C
Triglycerides (mg %)	77.6±31A	108±66A	111±6.1A	86.0±44A	115±12A	121±18A	82.2±46A	86.5±48A	4.6±50A
Glycerol (µg %)	379±166A	253±108ABC	315±143AB	79.3±66.2D	105±102CD	82.3±69D	82.0±96D	188±110BCD	92.3±31D
Total protein (g %)	1.3±0.29C	1.3±0.24C	1.4±0.29C	2.9±0.18AB	3.0±0.10A	2.8±0.10AB	2.8±0.05AB	2.9±0.1[illegible]AB	2.7±0.41B
Albumin (g %)	0.40±0.12C	0.35±0.10C	0.38±0.10C	1.1±0.08AB	1.2±0.10A	1.1±0.05AB	1.1±0.0AB	1.1±0.[illegible]A	1.1±0.06B
Albumin/globulin ratio	0.44±0.05B	0.39±0.05B	0.39±0.05B	0.61±0.06A	0.65±0.05A	0.69±0.08A	0.64±0.02A	0.62±0.05A	0.68±0.12A
Blood urea nitrogen (mg%)	1.0±0.82A	ND	1.0±1.4A	0.5±1.0A	ND	0.50±1.0A	0.25±0.50A	ND	0.25±0.50A
Uric acid (mg %)	6.8±0.83AB	5.6±1.0BC	5.7±0.77BC	7.7±1.2A	4.7±0.94C	6.8±1.7AB	6.2±1.6ABC	6.3±.66ABC	7.6±0.67A
Total bibirubin (mg %)	0.03±0.05A	0.03±0.05A	0.03±0.05A	0.03±0.05A	0.30±0.05A	0.05±0.06A	ND	ND	ND
Alkaline phosphatase (UL)	3500±0.0	3303±395	3500±0.0	3500±0.0	3483±125	3500±0.0	3500±0.0	3340±188	3445±110
LDH[d] (U/L)	771±119A	794±189A	831±119A	442±19B	440±21B	448±56B	398±46B	393±41B	412±68B
AST[d] (U/L)	165±16A	153±20AB	169±6.9A	132±10C	114±5.1C	129±18C	134±21BC	130±1.9C	160±13A
ALT[d] (U/L)	ND	ND	ND	ND	0.25±0.5	ND	ND	0.25±0.5	ND
SAL[d] (pmol/h/ul)	5.6±.54AB	5.1±.55ABC	5.1±.46ABC	4.7±.74BC	5.0±.30ABC	4.7±.44C	5.8±.58A	5.3±.32ABC	5.1±0.35A
GAL[d] (pmol/h/ul)	116±6.0AB	127±6.9A	110±11B	107±11B	115±6.9AB	104±9.1B	110±7.9B	110±10B	103±8.1B
Packed cell volume (%)	32.1±1.3CD	30.7±2.3D	32.8±2.5CD	32.8±0.63CD	32.5±1.4CD	31.6±1.6CD	35.8±2.2AB	33.9±.70BC	37.2±2.4A
Folic acid (ng/ml)	8.99±1.6A	9.26±0.64A	9.74±1.1A	5.25±1.9B	5.60±1.3B	6.20±3.1B	11.4±3.4A	5.92±0.34A	10.0±0.82A

[a]Values (means ± standard deviations) for a clinical parameter followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Pair fed to the feed intake of the corresponding aflatoxin group.

[c]Percentage of the NRC dietary requirement (1977) of threonine (basal ration provides 128%).

[d]TIBC - total iron binding capacity, LDH - lactate dehydrogenase, AST - aspartate aminotransferase, ALT - alanine aminotransferase, SAL - sialyltransferase, GAL - galactosyltransferase.

[e]ND - Not Detected.

App. 19a Major and interactive effects produced by graded levels of dietary threonine on the plasma biochemistry of chicks with aflatoxicosis.

Parameter (units)	Plasma parameter[a]								
	Effect of feeding			Effect of administration[d]			Anova [F (Pr>F)][f]		
	Aflatoxin[b]	Restricted[c]	Ad libitum	128	155	179	Feed	Admin	Feed x Admin
Calcium (mg%)	4.25C	6.69A	6.45B	A	A	A	2.21(0.0001)	0.22(0.80)	0.95(0.45)
Phosphorus, inorg. (mg%)	4.12B	4.74A	4.84A	A	A	A	46.1(0.0001)	0.25(0.78)	4.17(0.0090)
Total iron (μg%)	58.3B	114A	109A	A	A	A	41.5(0.0001)	1.04(0.37)	1.10(0.38)
TIBC[e] (μg%)	111B	175A	165A	142B	153AB	157A	77.3(0.0001)	4.03(0.029)	3.90(0.013)
Saturated transferin (%)	47.0B	66.5A	66.5A	A	A	A	11.5(0.0002)	1.56(0.23)	0.80(0.54)
Packed cell volume (%)	31.9B	32.3B	35.6A	A	A	A	14.9(0.0001)	2.46(0.10)	1.34(0.28)
Glucose (mg%)	260C	283A	271B	A	A	A	32.5(0.0001)	1.04(0.37)	1.70(0.18)
Cholesterol (mg%)	46.9C	132A	114B	93.9B	106A	92.8B	504(0.0001)	14.0(0.0001)	8.30(0.0002)
Triglycerides (mg%)	A	A	A	A	A	A	0.95(0.40)	1.13(0.34)	0.22(0.93)
Glycerol (μg%)	316A	88.8B	104B	A	A	A	17.1(0.0001)	0.42(0.66)	1.53(0.22)
Total-protein (g%)	1.30B	2.88A	2.78A	A	A	A	182(0.0001)	0.74(0.48)	0.66(0.63)
Albumin (g%)	0.38B	1.13A	1.08A	A	A	A	359(0.0001)	0.32(0.73)	0.81(0.53)
Globulin (g%)	0.93B	1.75A	1.70A	A	A	A	83.3(0.0001)	0.76(0.48)	0.76(0.56)
Albumin/globulin ratio	0.40B	0.65A	0.65A	A	A	A	56.6(0.0001)	0.62(0.55)	1.16(0.35)
Blood urea nitrogen (mg%)	A	A	A	A	A	A	1.35(0.28)	2.37(.11)	0.34(0.85)
Uric acid (mg%)	A	A	A	6.88A	5.54B	6.71A	1.15(0.33)	5.08(0.01)	2.74(0.049)
Total bilirubin (mg%)	A	A	A	A	A	A	2.05(0.15)	0.16(0.86)	0.16(0.96)
Alkaline phosphatase (U/L)	A	A	A	3500A	3360A	3482A	0.38(0.69)	2.86(0.075)	0.29(0.88)
LDH[e] (U/L)	798A	443B	401B	A	A	A	67.4(0.0001)	0.29(0.75)	0.10(0.98)
AST[e] (U/L)	162A	125C	141B	144AB	132AB	153A	21.0(0.0001)	6.48(0.0050)	1.24(0.32)
ALT[e] (U/L)	A	A	A	A	A	A	0.50(0.61)	2.00(0.16)	0.50(0.74)
Folic acid (ng/ml)	9.33A	9.10A	5.68B	8.54AB	6.93B	8.64A	14.1(0.0001)	3.15(0.059)	3.20(0.028)
Insulin (μg/ml)	16.8A	9.44B	14.9A	A	A	A	12.3(0.0002)	1.07(0.36)	1.42(0.25)
Glutathionine (nmol/gwwb)	306B	222C	472A	347A	238B	416A	19.8(0.0001)	98.2(0.0006)	12.0(0.0001)

[a]Means for a clinical parameter within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the feed intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of lysine (basal ration provided 128%).

[e]TIBC = total iron binding capacity, LDH = lactate dehydrogenase, AST = aspartate aminotransferase, ALT = alanine aminotransferase.

[f]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 29. Effect of graded levels of dietary lysine on the plasma biochemistry and hepatic glutathione of chicks with aflatoxicosis.

	Plasma parameter[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
Parameter (units)	102[c]	122[c]	146[c]	102	122	146	102	122	146
Calcium (mg %)	4.85±0.25C	4.50±0.22C	4.35±0.41C	6.33±0.54AB	6.25±0.61B	6.83±0.33A	6.45±0.62AB	6.68±0.26AB	6.80±0.3AB
Phosphorus, inorg. (mg %)	4.25±0.06B	4.23±0.25B	3.90±0.29B	5.70±0.44A	5.88±0.4A	5.73±0.49A	5.48±0.26A	5.63±0.15A	5.48±0.40A
Total iron (μg %)	74.3±12D	71.3±7.9D	62.2±9.9D	120±7.8ABC	118±18ABC	131±16AB	109±2.1C	136±33A	116±5.2BC
TIBC[d] (μg %)	106±26C	104±14C	88.5±8.2C	154±2.9AB	135±28B	161±21A	132±7.2B	174±32A	135±9.6B
Saturated transferin (%)	67.5±7.2C	67.5±5.7C	70.0±5.9C	77.8±4.5B	85.5±6.4A	82±4.5AB	83.0±3.4AB	77.8±4.9B	85.3±4.2A
Packed cell volume (%)	25.8±4.5B	26.5±1.3AB	27.3±2.5AB	29.0±2.5AB	31.0±0.82A	30±3.9AB	28.8±1.7AB	29±1.63AB	25.5±3.8B
Glucose (mg %)	262±5.4A	260±4.1A	268±6.0A	280±43A	286±25A	272±11A	271±4.4A	267±8.7A	265±8.8A
Cholesterol (mg %)	57.8±4.9C	55.5±9.9C	48.0±4.5C	120±9.0A	129±16A	129±10A	95.8±10B	102±7.4B	98.3±6.0B
Triglycerides (mg %)	67.0±20A	84.8±32A	109±13A	71.5±30A	108±20A	120±24A	89.7±44A	75.5±32A	84.3±34A
Glycerol (μg %)	463±186A	416±98AB	481±254A	101±84C	283±138ABC	278±137ABC	195±136BC	160±110C	178±147BC
Total protein (g %)	1.45±0.19B	1.18±0.15C	1.13±0.21C	2.45±0.15A	2.43±0.29A	2.50±0.12A	2.40±0.08A	2.43±0.10A	2.50±0.14A
Albumin (g %)	0.58±0.05C	0.58±0.10C	0.50±0.08C	1.15±0.[illegible]B	1.08±0.05B	1.18±0.05A	1.10±0.08AB	1.10±0.00AB	1.18±0.10A
Globulin (g %)	0.88±0.15B	0.60±0.08C	0.63±0.13C	1.30±0.08A	1.35±0.27A	1.33±0.10A	1.30±0.0A	1.33±0.10A	1.33±0.05A
Albumin/globulin ratio	0.67±0.08C	0.96±0.15A	0.80±0.04BC	.89±0.04AB	0.82±0.15B	0.89±0.07AB	.85±0.06AB	0.83±0.06AB	0.89±0.05A
Blood urea nitrogen (mg%)	1.83±0.57A	1.25±.5ABC	1.50±0.58AB	.28±0.49D	0.25±0.50D	0.78±0.45CD	1.00±0.0BC	1.00±0.0BC	1.00±0.0BC
Uric acid (mg %)	5.48±0.5AB	5.48±1.9AB	4.80±0.54B	5.95±1.4AB	6.30±0.57AB	6.30±2.5AB	7.38±0.34A	6.90±0.94AB	7.58±0.70A
Total bilirubin (mg %)	0.20±0.00A	0.20±0.00A	0.20±0.00A	0.18±0.05A	0.18±0.05A	0.18±0.05A	0.18±0.05A	0.20±0.00A	0.20±0.00A
Alkaline phosphatase (U/L)	3890±450A	4460±1100A	2200±630A	3000±1390A	3200±390A	4170±2300A	4380±1200A	4400±3000A	3070±471A
LDH[d] (U/L)	704±98AB	755±78A	650±90B	453±22CD	429±34CD	462±52C	410±32CD	385±19D	397±11CD
AST[d] (U/L)	157±15BC	148±15C	133±6.5D	202±3.5A	192±6.5A	193±12A	164±12B	163±9.4B	163±7.7B
ALT[d] (U/L)	0.0±0.0B	7.5±9.6AB	0.0±0.0B	2.5±5.0AB	0.0±0.0B	0.0±0.0B	0.0±0.0B	0.0±0.0B	10.0±12A
Folic acid (ng/ml)	9.68±2.8AB	8.68±1.4ABC	10.4±1.7A	8.80±1.2ABC	5.77±0.63C	7.48±2.8AB	8.3±0.66ABC	7.15±0.84BC	9.07±2.5AB
Insulin (μU/ml)	17.9±2.5A	16.0±5.4A	16.6±3.9A	13.1±5.8AB	8.52±2.9BC	6.73±2.7C	13.6±2.5AB	16.6±2.9A	14.5±3.6A
Glucagon (ng/ml)	31.5±15AB	26.3±9.9AB	2[illegible]±11B	23.5±14B	[illegible].3±20B	37.5±5.2A	35.5±2.1A	17.5±9.0B	22.8±16B
Glutathione (nmol/g wb)	257±97BC	320±46B	342±138B	152±21C	242±46BC	272±120BC	632±163A	151±90C	634±81A

[a] Values (means ± standard deviations) for a clinical parameter followed by the same letter are not significantly different at $P<0.05$.

[b] Pair fed to the feed intake of the corresponding aflatoxin group.

[c] Percentage of the NRC dietary requirement (1977) of lysine (basal ration provides 102%).

[d] TIBC = total iron binding capacity, LDH = lactate dehydrogenase, AST = aspartate aminotransferase, ALT = alanine aminotransferase.

App. 20a Major and interactive effects produced by graded levels of dietary lysine on the plasma biochemistry and hepatic glutathionine of chicks with aflatoxicosis.

Parameter (units)	Plasma parameter[a]: Effect of feeding: Aflatoxin[b]	Restricted[c]	Ad libitum	Effect of administration[d]: 102	122	146	Anova [F (Pr>F)][f]: Feed	Admin	Feed x Admin
Calcium (mg%)	4.57B	6.47A	6.64A	-A	-A	-A	88.6(0.0001)	0.58(0.57)	1.89(0.14)
Phosphorus, inorg. (mg%)	4.13B	5.77A	5.53A	-A	-A	-A	83.9(0.0001)	1.16(0.33)	0.39(0.81)
Total iron (µg%)	69.3B	123A	121A	-A	-A	-A	47.9(0.0001)	0.80(0.46)	2.13(0.11)
TIBC[e] (µg%)	99.4B	150A	147A	-A	-A	-A	32.4(0.0001)	0.99(0.38)	4.96(0.004)
Saturated transferin (%)	68.3B	81.8A	82.0A	-A	-A	-A	26.2(0.0001)	1.08(0.35)	1.73(0.17)
Packed cell volume (%)	26.5B	30.0A	27.8AB	-A	-A	-A	4.85(0.016)	0.67(0.52)	1.04(0.40)
Glucose (mg%)	-A	-A	-A	-A	-A	-A	2.53(0.099)	0.09(0.92)	0.41(0.80)
Cholesterol (mg%)	53.8C	126A	98.5B	-A	-A	-A	189(0.0001)	0.80(0.46)	1.08(0.39)
Triglycerides (mg%)	A	A	A	761B	894AB	1040A	1.09(0.35)	2.89(0.07)	1.26(0.31)
Glycerol (µg%)	446A	221B	178B	A	A	A	10.9(0.0003)	0.58(0.57)	0.77(0.55)
Total protein (g%)	1.25B	2.46A	2.44A	-A	-A	-A	207(0.0001)	0.93(0.41)	2.04(0.12)
Albumin (g%)	0.55B	1.13A	1.13A	-A	-A	-A	284(0.0001)	0.76(0.48)	2.35(0.079)
Globulin (g%)	0.70B	1.33A	1.32A	-A	-A	-A	97.4(0.0001)	1.12(0.34)	2.46(0.069)
Albumin/globulin ratio	-	-	-	-A	-A	-A	1.24(0.31)	2.34(0.12)	5.25(0.0029)
Blood urea nitrogen (mg%)	1.53A	0.43C	1.00B	-A	-A	-A	20.2(0.0001)	1.24(.30)	1.31(0.29)
Uric acid (mg%)	5.25B	6.18B	7.28A	-A	-A	-A	8.05(0.0018)	0.00(0.10)	0.40(0.81)
Total bilirubin (mg%)	-	-	-	-A	-A	-A	1.75(0.19)	0.25(0.78)	0.25(0.91)
Alkaline phosphatase (U/L)	-	-	-	-A	-A	-A	0.39(0.68)	1.11(0.34)	1.58(0.21)
LDH[e] (U/L)	703A	448B	397C	-A	-A	-A	98.1(0.0001)	0.47(0.63)	1.71(0.18)
AST[e] (U/L)	146C	196A	163B	174A	168AB	163B	68.5(0.0001)	3.49(0.045)	1.61(0.20)
ALT[e] (U/L)	-	-	-	-A	-A	-A	0.70(0.51)	0.70(0.51)	3.55(0.019)
Folic acid (ng/ml)	9.59A	7.35B	8.19AB	8.94A	7.20B	8.98A	4.73(0.017)	3.84(0.034)	0.55(0.70)
Insulin (µg/ml)	16.8A	9.44B	14.9A	-A	-A	-A	12.3(0.0002)	1.07(0.36)	1.42(0.25)
Glutathionine (nmol/g wb)	306B	222C	472A	347A	238B	416A	19.8(0.0001)	9.82(0.0006)	12.0(0.0001)

[a]Means for a clinical parameter within a major effect followed by the same letter are not significantly different at $P<0.05$.

[b]Dietary aflatoxin = 2.5µg per g of diet.

[c]Pair-fed to the intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of lysine (basal ration provided 102%).

[e]TIBC = total iron binding capacity, LDH = lactate dehydrogenase, AST = aspartate aminotransferase, ALT = alanine aminotransferase.

[f]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 21. Effect of dietary or interperitoneal administration (IP) of choline on concentrations of free-amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

FAA or NPS	Free-amino acid or ninhydrin-positive substance (nmol/ml of plasma)[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
	0[c]	IP[d]	Dietary[d]	0	IP	Dietary	0	IP	Dietary
Alanine	612±48AB	503±68CDE	542±55DE	457±39DE	441±73E	549±37BCD	620±91AB	659±135A	604±21ABC
γ-Aminobutyric acid	13±1.4B	7.6±5.0CD	3.1±0.9D	3.9±1.1CD	5.8±4.5CD	5.6±3.2CD	9.1±5.3BC	14±6.4B	24±3.2A
α-Amino-n-butyric acid	16±1.5BC	17±4.0BC	14±0.90C	19±2.5B	17±3.5Bc	42±8.7A	13±1.2C	16±3.9BC	15±1.3BC
Ammonia	272±25B	237±54B	270±60B	283±34B	214±5.2B	279±40B	282±94B	263±107B	431±18A
Arginine	517±49B	463±50C	569±51A	303±51D	28±28D	252±18D	498±38BC	487±31BC	495±26BC
Asparagine	152±71CD	123±23CD	170±40C	119±3.0D	64±19E	107±27DE	278±26AB	256±36B	308±32A
Aspartic Acid	78±20AB	85±29A	82±23AB	37±7.4D	65±4.1ABC	34±3.0D	50±13CD	63±6.2BC	35±2.5D
Carnosine	17±5.1	15±9.6	Tr±0.0	6.5±9.2	4.5±6.4	ND[e]	ND	ND	5.0±8.7
Citrulline	6.9±1.3AB	4.5±1.6ABC	2.8±1.0C	8.0±1.7A	4.0±1.6C	4.7±1.6ABC	5.4±3.8ABC	5.3±1.8ABC	5.6±2.7AB
Cystathionine	8.0±2.2E	8.1±0.5E	9.6±1.0DE	10±2.0CDE	12±2.6BC	8.4±0.7E	13±3.1B	17±1.0A	11±1.0BC
Cysteic acid/phosphoserine	19±2.9C	17±1.7C	20±1.7C	25±3.5B	21±2.7C	27±42B	31±1.0A	22±2.2C	27±3.6AB
Half-cystine	54±13D	62±9.4CD	72±15BC	80±11B	72±6.4BC	61±8.9CD	95±8.3A	105±8.3A	95±5.5A
Ethanolamine	27±7.8A	23±4.7B	17±3.8B	5.7±6.6CDE	0.10±0.0E	15±1.9BC	12±8.5BCD	2.9±5.7DE	16±2.7B
Glutamic acid	123±11BCD	114±9.0BCD	119±6.6BCD	112±11D	126±9.6B	114±2.5BCD	143±9.5A	148±9.3A	124±8.4BC
Glutamine	1098±85A	909±55BC	1057±98AB	617±133F	640±44DE	594±129E	800±211CD	898±173BC	749±64CDE
Glycine	739±48BC	690±79C	723±30BC	522±105D	482±26D	494±69D	814±119AB	799±64AB	835±23A
Histidine	151±14AB	133±9.1B	168±7.4A	69±10CD	72±6.7C	54±8.3D	163±22A	169±26A	135±7.2B
Hydroxylysine	2.4±1.5ABC	1.2±0.3C	1.8±0.62ABC	2.4±1.0ABC	1.2±0.1C	3.3±1.7A	3.1±1.7AB	1.4±0.6BC	2.6±0.70A
Hydroxyproline	118±14C	105±7.0C	113±17C	155±14B	146±34B	156±16B	209±9.2A	189±12A	197±6.5A D
Isoleucine	123±17C	107±8.7CDE	148±5.8B	103±5.3DE	97±4.2E	122±13CD	172±22A	182±26A	164±12AB
Leucine	245±29BC	213±20CD	258±25B	200±12D	181±20D	210±22CD	326±35A	342±49A	308±19A
Lysine	336±53B	344±78B	354±34B	403±34B	358±28B	515±64A	568±61A	552±59A	570±18A
Methionine	60±6.5BC	65±6.4B	69±6.4B	54±5.9CD	47±3.8D	65±7.2B	90±11A	96±11A	93±5.1A
1-Methylhistidine	12±7.6A-D	16±4.8ABC	17±6.8AB	10±5.3BCD	13±2.1A-D	6.0±3.0D	11±6.9A-D	20±5.0A	6.9±2.9CD
3-Methylhistidine	17±2.9ABC	17±5.4AB	19±4.8A	12±2.2C	13±1.2BC	19±2.5A	13±2.5BC	15±1.9ABC	16±1.8AB
Ornithine	90±9.3B	66±9.7C	113±15A	29±8.2D	27±10D	23±6.4D	110±15A	119±19A	106±10A
Phenylalanine	272±63A	247±83A	290±94A	102±18B	91±21B	96±4.4B	150±16B	156±15B	139±4.0B
Proline	575±54BC	542±68C	543±27C	325±58D	264±44D	272±52D	608±63ABC	629±42AB	675±72A
Serine	599±45D	600±107D	661±33CD	747±111BC	793±11AB	886±69A	644±93CD	745±124CB	785±28AB
Taurine	291±14A	226±142AB	205±30ABC	115±18CD	109±12D	220±51AB	161±22BCD	159±73BCD	177±42BCD
Threonine	412±73C	465±110C	468±44C	571±115C	529±73C	533±83C	824±63B	987±257A	816±98B
Tryptophan	61±5.8CD	49±13E	59±5.1CDE	62±7.8C	60±5.3CD	52±6.4DE	101±8.5A	100±6.6A	85±4.6B
Tyrosine	236±14A	215±42EF	216±25AB	145±16EF	141±4.0F	166±14DEF	197±0.4BC	190±7.9CD	170±8.4DE
Valine	264±33C	240±20CD	323±10B	206±15DE	186±24E	219±22DE	364±38A	383±42A	357±8.3AB

[a] Values (means ± SEM) for FAA or NPS followed by the same letter are not significantly different at P ≤0.05.
[b] Pair fed to the feed intake of the corresponding aflatoxin group.
[c] No supplemental choline.
[d] Administration to achieve 175% of the NRC requirement (1977) of choline (basal ration provides 106%)
[e] ND = Not detectable at levels less than (a 0.1nmol/ml: β-alanine, α-aminoadipic acid, β-aminoisobutyric acid, anserine, galactosamine, glucosamine, glycerophosphoethanolamine, homocitrulline, homocystine, methionine sulfone or sulfoxide, phosphoethanolamine, sarcosine.

App. 21a. Major and interactive effects produced by dietary or intraperitoneal administration of choline on concentrations of free-amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

	FAA or NPS (nmol/mL)[a]						ANOVA (F[Pr> F])[f]		
	Effect of feeding			Effect of administration					
	Aflatoxin[b]	Restricted[c]	Ad libitum	0[d]	IPe	Dietary[e]	Feed	Admin	Feed x Admin
Alanine	353B	482C	617A	-A	-A	-A	12.7(0.0001)	0.69(0.51)	2.55(0.062)
α-Amino-n-butyric acid	15.4B	25.9A	14.3B	15.7A	10.3B	23.6A	34.1(0.0001)	15.7(0.0001)	20.2(0.0001)
Ammonia	260B	239B	325A	279B	238B	327A	5.87(0.013)	7.02(0.0035)	2.64(0.059)
Arginine	316A	268B	493A	439A	400B	439A	142(0.0001)	3.72(0.037)	2.82(0.045)
Asparagine	148B	96.8C	281A	183A	148B	195A	80.5(0.0001)	5.33(0.011)	0.40(0.81)
Aspartic acid	81.6A	45.1B	49.3B	55.1B	70.7A	50.2B	20.7(0.0001)	6.01(0.0070)	1.37(0.27)
Citrulline	-A	-A	-A	6.78A	4.60B	4.38B	0.55(0.58)	5.50(0.014)	1.74(0.17)
Cystathionine	8.57C	10.2B	13.7A	10.4B	12.3A	9.73B	2.56(0.0001)	6.54(0.0048)	3.98(0.012)
Cysteic acid/phosphoserine	26.3A	24.1B	18.7C	2.52A	19.3B	24.6A	23.4(0.0001)	15.8(0.0001)	2.68(0.053)
Half-cystine	62.4C	71.1B	98.2A	-A	-A	-A	41.0(0.0001)	0.60(0.56)	3.88(0.013)
Ethanolamine	20.5A	6.81B	10.3B	14.8A	6.83B	1.60A	14.3(0.0001)	700(0.0036)	2.64(0.055)
Glutamic acid	119B	117B	138A	126AB	130A	119B	21.5(0.0001)	4.33(0.023)	4.05(0.011)
Glutamine	1021A	617C	815B	-A	-A	-A	32.8(0.0001)	0.30(0.74)	2.01(0.12)
Glycine	718B	500C	816A	-A	-A	-A	62.8(0.0001)	0.79(0.47)	0.15(0.96)
Histidine	151A	65.0B	155A	-A	-A	-A	159(0.0001)	1.21(0.31)	6.87(0.0006)
Hydroxylysine	-A	-A	-A	2.63B	1.22B	2.55A	0.99(0.38)	6.75(0.0042)	0.86(0.50)
Hydroxyproline	112C	152B	198A	-A	-A	-A	85.4(0.0001)	2.10(0.14)	0.17(0.95)
Isoleucine	126B	107C	172A	132AB	129B	144A	62.7(0.0001)	3.82(0.035)	4.45(0.0068)
Leucine	238B	197C	325A	-A	-A	-A	68.0(0.0001)	0.81(0.46)	2.34(0.080)
Lysine	344C	423B	563A	436AB	418B	479A	55.9(0.0001)	4.60(0.019)	2.81(0.045)
Methionine	65.0B	55.3C	92.8A	68.2B	69.2AB	75.7A	81.6(0.0001)	3.57(0.042)	2.18(0.098)
1-Methylhistidine	14.9A	9.48B	12.6AB	11.0B	15.9A	10.1B	3.16(0.059)	4.16(0.027)	2.31(0.084)
3-Methylhistidine	17.7A	14.7B	14.9A	14.0B	15.2B	18.1A	3.52(0.044)	5.69(0.0087)	0.82(0.53)
Ornithine	89.7B	26.3C	112A	-A	-A	-A	161(0.0001)	2.06(0.15)	7.28(0.0004)
Phenylalanine	270A	96.6C	148B	-A	-A	-A	40.9(0.0001)	0.18(0.83)	0.42(0.79)
Proline	553B	287C	637A	-A	-A	-A	133(0.0001)	0.64(0.54)	1.44(0.25)
Serine	620C	809A	725B	663B	713AB	777A	17.2(0.0001)	6.25(0.0059)	0.56(0.70)
Taurine	241A	148B	165B	-A	-A	-A	8.20(0.0017)	1.14(0.33)	2.84(0.044)
Threonine	448C	545B	876A	-A	-A	-A	43.7(0.0001)	0.92(0.41)	1.11(0.37)
Tryptophan	56.2B	57.8B	95.1A	74.6A	69.4AB	65.0B	105(0.0001)	5.01(0.014)	2.89(0.041)
Tyrosine	222A	151C	186B	-A	-A	-A	43.8(0.0001)	1.08(0.36)	2.39(0.076)
Valine	276B	204C	368A	278AB	270B	300A	119(0.0001)	4.21(0.026)	4.54(0.0062)

[a]Means for FAA or NPS within a major effect followed by the same letter are not significantly different at P≤0.05.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the food intake of the corresponding aflatoxin group.

[d]No supplemented choline.

[e]Administration to achieve 175% of NRC requirement (1977) of choline (basal ration provided 106%).

[f]Admin = Major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 22. Effect of graded levels of dietary folic acid on concentrations of free-amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

FAA or NPS	Free-amino acid or ninhydrin-positive substance (nmole/ml of plasma)[a]								
	Aflatoxin (2.5 μg/g of diet)			Restricted Feeding[b]			Control		
	244[c]	344[c]	644[c]	244	344	644	244	344	644
Alanine	359±38C	484±58C	524±16C	755±63B	934±68A	936±120A	737±81B	774±141B	699±36B
γ-Aminobutyric acid	2.1±2.3A	4.0±2.3A	5.5±4.4A	2.9±0.78A	4.0±2.1A	5.3±1.5A	5.0±2.7A	2.4±0.45A	4.5±1.9A
α-Amino-n-butyric acid	9.4±4.6EF	13±1.5DE	8.1±2.2F	21±2.9AB	20±1.5AB	22±2.9A	18±5.9ABC	15±1.5CD	17±2.1BCD
Ammonia	252±14AB	291±78A	277±12AB	235±63AB	244±47AB	271±46AB	193±44B	202±68AB	211±38B
Asparagine	99±19C	114±47C	101±19C	193±55B	216±25B	285±43A	210±17B	182±4.5B	176±19B
Aspartic acid	129±12A	122±2.1AB	109±6.8B	51±4.2D	62±8.2CD	59±6.5CD	56±14D	50±6.2D	70±20C
Arginine	530±22A	530±42A	548±77A	494±42A	539±21A	551±41A	465±80A	520±51A	491±76A
Citrulline	5.0±1.4BCD	4.1±0.70BCD	5.3±2.2BC	6.2±2.6B	15±0.86A	3.9±1.3CD	5.5±2.4BC	3.0±0.60D	6.3±1.3B
Cystathionine	5.3±2.1E	7.1±2.5DE	4.3±0.76E	13±1.9AB	15±2.2AB	16±3.7A	13±1.5BC	9.8±1.3CD	9.8±2.9CD
Cysteic acid/phosphoserine	28±3.7A	24±3.6AB	25±2.2A	21±2.7BC	26±4.2A	27±2.1A	17±1.6C	19±2.8C	21±2.3BC
Half-cystine	90±6.2C	87±11C	93±12C	97±5.7BC	116±8.7A	112±7.3A	94±9.3C	94±6.0C	109±7.0AB
Ethanolamine	16±7.9A	17±9.2A	16±6.0A	18±2.2A	13±1.0A	20±4.0A	13±1.4A	14±3.1A	15±5.2A
Glutamic acid	123±18A	127±12AB	113±12B	134±13AB	137±11AB	133±1.4AB	136±24AB	142±7.7A	145±11A
Glutamine	1120±110A	982±111BC	1071±31AB	733±67E	1132±82A	1093±92AB	869±40D	796±100DE	887±75CD
Glycine	720±51D	705±19D	740±59D	851±79BC	920±91B	1066±33A	790±24CD	725±127D	726±20D
Histidine	128±6.5CD	120±11CD	142±10BC	160±16B	193±29A	189±18A	118±17CD	108±27D	124±13CD
Hydroxylysine	3.9±0.25AB	3.1±1.0ABC	4.0±0.00A	2.5±0.14C	2.6±0.43C	3.0±0.61BC	2.7±0.93C	2.5±0.41C	3.4±.70ABC
Hydroxyproline	164±18D	170±20CD	170±43CD	208±4.0AB	202±9.9BC	220±7.5AB	194±33BCD	170±30CD	239±17A
Isoleucine	104±11BC	115±15ABC	98±9.8C	121±5.8AB	126±7.6A	131±15A	124±12A	119±17AB	124±16A
Leucine	190±16CD	212±25C	181±15D	243±12B	274±11A	263±22AB	252±8.2AB	241±24B	242±9.5B
Lysine	206±84CD	231±39BCD	164±38D	279±52ABC	179±45D	283±27ABC	363±71A	304±90AB	335±57A
Methionine	62±13CD	59±8.6D	57±5.1D	79±9.2CB	82±13B	103±30A	84±7.1AB	76±7.2BCD	85±5.4AB
1-Methylhistidine	13±1.7CD	9.2±0.66E	15±0.49C	16±1.9BC	19±2.1AB	20±3.7A	10±4.3DE	15±3.3C	14±3.1C
3-Methylhistidine	10±6.2D	14±3.4BCD	12±1.8CD	20±5.2A	14±0.83BCD	18±1.6AB	17±1.8ABC	14±1.8BCD	17±2.7AB
Ornithine	41±6.1CD	71±3.6A	40±0.92	71±7.7A	54±14BC	67±14AB	65±16AB	49±7.4CD	46±6.5CD
Phenylalanine	208±34B	242±17A	208±28B	114±8.0C	135±8.7C	128±11C	133±15C	127±18C	138±6.1C
Proline	517±22CD	490±49D	548±27CD	781±66B	925±94A	920±81A	572±75CD	582±95C	560±65CD
Serine	561±30EF	479±80F	618±34DE	756±50C	888±66B	1034±83A	727±104C	725±110C	673±45CD
Taurine	235±25A	257±37A	169±51B	101±18C	109±12C	100±17C	139±44BC	110±29C	123±17C
Threonine	336±31D	322±38D	305±38D	615±130BC	692±118AB	738±40A	596±86BC	540±119C	647±40ABC
Tryptophan	54±13BC	66±3.9B	50±6.5C	86±2.4A	79±9.0A	83±13A	84±3.9A	82±5.0A	81±10A
Tyrosine	228±19A	192±30B	229±31A	125±7.8D	156±12CD	130±8.9D	180±17BC	151±31CD	191±29B
Valine	226±30BC	256±30ABC	222±20C	258±11ABC	295±49A	274±31A	261±24ABC	268±22AB	255±8.2ABC

[a] Values (means ± SEM) for FAA or NPS followed by the same letter are not significantly different at $P \leq 0.05$ ND = Not detectable at levels less than ca 0.1nmol/ml: α-aminoadipic acid, β-aminoisobutyric, γ-aminobutyric acid, anserine, carnosine, glucosamine, glycerophosphoethanolamine, homocitrulline, homocystine, methionine sulfone or sulfoxide, phosphoethanolamine.
[b] Pair fed to the feed intake of the corresponding aflatoxin group.
[c] Percentage of the NRC dietary requirement (1977) of folate (basal ration provides 244%).

App. 22a. Major and interactive effects produced by graded levels of dietary folic acid on concentrations of free-amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

	FAA or NPS (nmol/ml)[a]						ANOVA (F(Pr > F))[e]		
	Effect of feeding			Effect of administration[d]					
	Aflatoxin[b]	Restricted[c]	Ad libitum	[illegible]	[illegible]	[illegible]	Feed	Admin	Feed x Admin
Alanine	332C	475A	737B	-A	-A	-A	61.2(0.0001)	1.18(0.32)	3.83(0.014)
α-Amino-n-butyric acid	10.1C	20.9A	16.5B	-A	-A	-A	36.3(0.0001)	0.06(0.94)	2.06(0.11)
Ammonia	273A	250A	202B	-A	-A	-A	6.33(0.0036)	0.88(0.43)	0.21(0.93)
Arginine	-A	-A	-A	-A	-A	-A	2.24(0.13)	1.35(0.23)	0.43(0.79)
Asparagine	105C	231A	189B	-A	-A	-A	50.0(0.0001)	1.32(0.28)	4.66(0.0055)
Aspartic Acid	120A	37.0B	38.3B	-A	-A	-A	1.50(0.0001)	0.08(0.92)	4.66(0.0055)
Citrulline	4.78B	8.30A	4.91B	5.54B	7.31A	5.12B	17.8(0.0001)	5.99(0.0070)	24.8(0.0001)
Cystathionine	3.55C	14.7A	10.7A	-A	-A	-A	49.6(0.0001)	0.26(0.78)	2.51(0.065)
Cysteic acid/phosphoserine	25.7A	24.5A	19.0B	[illegible]	-A	-A	18.0(0.0001)	2.16(0.13)	3.28(0.026)
Half-cystine	89.9C	108A	98.7B	93.7B	98.7AB	105A	14.7(0.0001)	5.03(0.016)	2.64(0.055)
Ethanolamine	-A	-A	-A	-A	-A	-A	0.94(0.40)	0.39(0.56)	0.66(0.62)
Glutamic acid	121B	135A	141A	-A	-A	-A	7.01(0.0035)	0.46(0.64)	0.63(0.65)
Glutamine	1056A	986B	850C	907B	970AB	1017A	19.3(0.0001)	3.37(0.012)	13.5(0.0001)
Glycine	722B	945A	747B	787AB	783B	844A	41.4(0.0001)	3.18(0.058)	4.69(0.0053)
Histidine	130B	181A	117B	135A	140A	151A	48.8(0.0001)	2.56(0.096)	1.92(0.14)
Hydroxylysine	3.67A	2.68B	2.84B	3.03AB	2.72B	3.44A	9.43(0.0008)	4.47(0.021)	0.60(0.67)
Hydroxyproline	168B	210A	201A	189B	181B	210A	108(0.0004)	4.93(0.015)	2.32(0.082)
Isoleucine	106B	126B	122B	-A	-A	-A	10.5(0.0004)	0.35(0.70)	1.62(0.19)
Leucine	194C	260A	245B	228A	242A	229A	49.3(0.0001)	2.60(0.092)	2.36(0.079)
Lysine	200C	247B	334A	-A	-A	-A	15.6(0.0001)	1.70(0.20)	2.27(0.088)
Methionine	59.3B	88.0A	81.5A	-A	-A	-A	16.2(0.0001)	1.55(0.23)	1.52(0.23)
1-Methylhistidine	17.5B	18.6A	13.0B	15.2B	14.4AB	16.5A	19.7(0.0001)	4.55(0.020)	3.32(0.025)
3-Methylhistidine	12.1B	17.2A	16.1A	-A	-A	-A	8.02(0.0018)	0.87(0.43)	2.35(0.079)
Ornithine	50.5B	63.9A	53.3B	59.1A	58.0A	50.7A	6.22(0.0060)	2.61(0.092)	9.48(0.0001)
Phenylalanine	[illegible]	126B	133B	-A	-A	-A	95.8(0.0001)	2.48(0.10)	1.87(0.15)
Proline	518C	876A	571B	-A	-A	-A	95.3(0.0001)	1.98(0.16)	2.25(0.090)
Serine	553C	992A	708B	691B	679B	775A	66.2(0.0001)	5.76(0.0082)	6.73(0.0007)
Taurine	220A	103B	124B	158A	158A	130B	58.0(0.0001)	3.35(0.050)	3.36(0.024)
Threonine	321C	682A	594B	-A	-A	-A	64.6(0.0001)	1.30(0.29)	1.45(0.25)
Tryptophan	56.7B	82.2A	82.4A	-A	-A	-A	39.0(0.0001)	0.82(0.45)	1.90(0.14)
Tyrosine	216A	137C	174B	-A	-A	-A	36.4(0.0001)	1.75(0.19)	3.57(0.018)
Valine	335B	276A	262A	248A	273A	250A	7.00(0.0036)	2.99(0.067)	0.43(0.78)

[a]Means for FAA or NPS within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the food intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of folic acid (basal ration provided 244%).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 23. Effect of graded levels threonine on concentrations of free-amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

FAA or NPS	Free-amino acid or ninhydrin-positive substance (nmol/ml of plasma)[a]								
	Aflatoxin (2.5 µg/g of diet)			Restricted Feeding[b]			Control		
	128[c]	155[c]	179[c]	128	155	179	128	155	179
Alanine	583±29AB	465±19CD	521±43BC	551±50B	437±48D	512±91CD	520±84BC	650±9.9A	638±80A
α-Amino-n-butyric acid	11±2.3AB	10±3.4B	11±2.2AB	13±1.9AB	16±3.5AB	13±4.9AB	12±3.4AB	18±6.8A	10±4.1AB
Ammonia	350±77A	325±41A	342±26A	201±17B	238±43B	229±17B	235±38B	217±21B	235±34B
Arginine	553±46A	532±53A	502±24AB	439±67BC	348±16DE	348±62E	351±68DE	422±26C	414±45CD
Asparagine	293±17A	241±50B	322±30A	90±5.2D	99±16D	94±14D	98±21D	94±7.9D	132±30C
Aspartic Acid	69±42CD	83±36BCD	29±50D	242±24A	116±48BC	138±42B	131±20B	205±21A	258±53A
Citrulline	4.3±0.96BC	2.8±0.50CD	3.5±1.7BCD	6.2±0.45A	3.3±1.8BCD	2.6±0.8CD	2.3±0.47D	5.0±20AB	4.0±1.2BCD
Cystathionine	8.1±0.5EF	7.8±1.9EF	7.3±1.2F	14±2.1B	17±2.9A	16±1.7A	11±1.2CD	12±1.1BC	9.7±1.6ED
Cysteic acid/phosphoserine	14±2.5BC	15±3.4B	19±4.2A	10±1.1D	11±2.2BCD	12±2.2BCD	11±0.7CD	11±1.3CD	8.8±0.97D
Half-cystine	97±4.0CD	94±7.3CD	92±3.6D	115±6.3AB	116±3.8A	114±8.3AB	105±16BC	117±3.4A	104±8.3BC
Ethanolamine	12±1.8A	9.1±5.9A	10±1.4A	14±4.0A	14±4.0A	8.4±1.6A	13±4.6A	12±3.2A	7.3±3.4A
Glutamic acid	161±18AB	158±22AB	163±16AB	133±15B	172±21A	167±23A	175±13A	152±3.8AB	157±14AB
Glutamine	925±28A	898±38AB	905±37AB	838±27ABC	729±44D	838±126ABC	770±66CD	837±48BC	827±38BC
Glycine	846±78ABC	827±82BC	798±74BC	884±101ABC	630±84D	836±149ABC	740±97CD	980±53A	899±127AB
Histidine	164±5.2A	132±22BC	133±9.6BC	166±30A	107±12C	128±33BC	122±14BC	146±7.4AB	140±11AB
Hydroxylysine	1.8±1.2A	2.5±0.58A	2.7±0.83A	2.9±1.3A	1.4±0.3A	1.2±0.24A	2.1±1.4A	1.9±1.7A	2.4±0.95A
Hydroxyproline	349±33AB	207±78C	318±45ABC	218±14BC	249±29ABC	266±60ABC	307±127ABC	272±18ABC	372±147A
Isoleucine	163±9.5A	140±14AB	132±15B	140±19AB	102±17C	124±22BC	126±24BC	128±13B	139±11AB
Leucine	296±15A	251±26AB	238±30AB	269±29AB	207±27B	284±80A	249±37AB	253±24AB	272±21AB
Lysine	406±23AB	281±54CD	239±41D	405±34AB	226±42D	339±39BC	456±75A	419±78A	394±80AB
Methionine	54±5.3CD	52±8.6DC	46±2.0D	73±9.8A	54±5.0CD	61±8.7BC	60±9.8BC	68±6.2AB	61±4.8BC
1-Methylhistidine	9.4±1.8B	7.3±1.2B	7.7±3.0B	8.4±20B	9.0.1.2B	9.4±2.9B	8.4±1.7B	14±1.8B	7.9±2.5B
3-Methylhistidine	21±5.4A	19±0.90AB	17±1.1BC	14±2.0C	14±1.5C	14±1.1C	17±1.7BC	19±2.3AB	16±2.8BC
Ornithine	133±5.3A	95±12B	86±0.73BC	100±21B	38±77E	52±22DE	56±20DE	70±14CD	76±4.6C
Phenylalanine	228±49B	269±86AB	323±56A	131±15C	90±12C	122±32C	127±21C	108±30C	142±14C
Proline	611±34AB	510±36BC	492±10BC	654±116A	444±92C	492±119BC	440±98C	574±36AB	569±52AB
Serine	603±55EF	627±83EF	572±37F	732±83CD	850±50AB	928±141A	696±33DE	836±25AB	818±93BC
Taurine	340±68A	342±52A	332±37A	145±45B	156±23B	129±24B	180±19B	339±72A	173±15B
Threonine	648±51C	850±193C	911±46C	864±117C	1544±369B	1889±247A	849±195C	1810±375AB	2105±237A
Tryptophan	66±50DE	61±4.2E	64±6.1E	82±8.3AB	79±6.3ABC	73±6.1CD	75±7.3BC	86±65.A	86±4.0A
Tyrosine	224±26A	205±15AB	215±19A	128±27DE	102±14E	136±24D	167±18C	151±26CD	178±7.5BC
Valine	352±19A	295±14B	283±19B	304±41B	187±27C	211±55C	260±46B	273±24B	274±24B

[a]Values (means ± standard deviations) for FAA or NPS followed by the same letter are not significantly different at P<0.05
ND - Not detectable at levels less than ca 0.1nmol/ml: α-aminoadipic acid, anserine, γ-aminobutyric acid, β-aminoisobutyric acid, carnosine, glucosamine, glycerophosphoethanolamine, homocitrulline, homocystine, methionine sulfone or sulfoxide, phosphoethanolamine, sarcosine.
[b]Pair fed to the feed intake of the corresponding aflatoxin group.
[c]Percentage of the NRC dietary requirement (1977) of threonine (basal ration provides 128%).

App. 13a. Major and interactive effects produced by graded levels of threonine on concentrations of free-amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

FAA or NPS	Free-amino acid or ninhydrin positive substances (nmol/ml of plasma)[a]						ANOVA [F(Pr > F)][e]		
	Effect of feeding			Effect of administration[d]					
	Aflatoxin[b]	Restricted[c]	Ad libitum	128	153	179	Feed	Admin	Feed x Admin.
Alanine	523B	500B	602A	-A	-A	-A	1056(0.0004)	1.67(0.21)	8.46(0.005)
α-Amino-n-butyric acid	10.6A	14.0A	13.3A	-A	-A	-A	2.37(0.085)	2.32(0.12)	1.28(0.30)
Ammonia	339A	223B	229B	-A	-A	-A	33.7(0.0001)	0.17(0.85)	0.73(0.56)
Arginine	529A	378B	396B	-A	-A	-A	34.2(0.0001)	0.87(0.43)	3.73(0.02)
Asparagine	60C	163B	198A	-A	-A	-A	35.9(0.0001)	0.26(0.78)	10.6(0.0001)
Aspartic acid	295A	943B	100B	160B	145B	182A	219(0.0001)	7.11(0.0033)	3.38(0.013)
Carnosine									
Citrulline	-A	-A	-A	-A	-A	-A	0.55(0.58)	1.69(0.20)	7.22(0.0004)
Cystathionine	7.69C	15.4A	10.8B	10.7B	12.2A	11.0AB	62.1(0.0001)	2.84(0.076)	1.75(0.17)
Cysteic acid/phosphoserine	15.6A	11.0B	10.0B	-A	-A	-A	19.5(0.0001)	1.22(0.31)	2.39(0.075)
Half-cystine	94.3C	115A	109B	-A	-A	-A	22.7(0.0001)	1.68(0.21)	1.18(0.34)
Ethanolamine	-A	-A	-A	17.6A	11.5AB	8.63B	0.67(0.52)	3.89(0.033)	0.97(0.04)
Glutamic acid	-A	-A	-A	-A	-A	-A	0.21(0.81)	0.36(0.70)	3.92(0.012)
Glutamine	909A	802B	811B	-A	-A	-A	12.5(0.0001)	1.16(0.33)	2.64(0.056)
Glycine	-A	-A	-A	-A	-A	-A	2.54(0.098)	0.33(0.72)	6.09(0.0006)
Histidine	-A	-A	-A	151A	128B	134B	0.87(0.43)	4.68(0.018)	5.58(0.0021)
Hydroxylysine	-A	-A	-A	-A	-A	-A	0.66(0.52)	0.27(0.77)	1.98(0.13)
Hydroxyproline	291AB	245B	317A	291AB	243B	319A	2.79(0.079)	3.07(0.063)	1.40(0.24)
Isoleucine	145A	122B	131B	143A	124B	132AB	5.06(0.0077)	4.16(0.027)	2.79(0.046)
Leucine	-A	-A	-A	271A	237B	263AB	0.15(0.86)	2.91(0.072)	2.37(0.061)
Lysine	309B	324B	423A	472A	309B	324B	15.1(0.0001)	15.8(0.0001)	3.44(0.02)
Methionine	50.6B	62.5A	62.0A	-A	-A	-A	11.4(0.0003)	2.21(0.13)	4.00(0.011)
1-Methylhistidine	8.14A	8.93A	10.1A	-A	-A	-A	2.56(0.096)	2.23(0.13)	4.55(0.006)
3-Methylhistidine	19.0A	13.7B	17.3A	-A	-A	-A	14.8(0.0001)	1.85(0.18)	1.63(0.20)
Ornithine	105A	63.4B	67.4B	96.4A	67.7B	71.3B	31.4(0.0001)	14.9(0.0001)	10.8(0.0001)
Phenylalanine	273A	115B	126B	162AB	156B	196A	51.7(0.0001)	3.16(0.058)	1.85(0.15)
Proline	-A	-A	-A	-A	-A	-A	0.06(0.95)	2.08(0.14)	6.50(0.0008)
Serine	601B	837A	783A	677B	771A	773A	32.5 (0.0001)	6.43(0.0052)	2.59(0.059)
Taurine	338A	143C	231B	222B	279A	211B	58.9(0.0001)	8.16(0.0017)	5.18(0.0032)
Threonine	803B	1432A	1388A	787C	1401B	1635A	38.3(0.0001)	42.6(0.0001)	5.42(0.0025)
Tryptophan	63.7B	78.1A	82.4A	-A	-A	-A	30.7(0.0001)	0.03(0.97)	3.74(0.015)
Tyrosine	214A	122C	165B	173A	153B	176A	61.5(0.0001)	4.75(0.017)	0.47(0.76)
Valine	318A	234C	269C	305A	252B	256B	16.1(0.0001)	9.07(0.0006)	4.01(0.0044)

[a] Means for FAA or NPS within a major effect followed by the same letter are not significantly different at P<0.05.

[b] Dietary aflatoxin = 2.5μg per g of diet.

[c] Pair-fed to the food intake of the corresponding aflatoxin group.

[d] Percentage of the NRC dietary requirement (1977) of threonine (basal ration provided).

[e] Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 24. Effect of graded levels of dietary lysine on concentrations of free-amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

FAA or NPS	Free-amino acid or ninhydrin-positive substance (nmol/ml of plasma)[a] Aflatoxin (2.5 µg/g of diet)			Restricted Feeding[b]			Control		
	102[c]	122[c]	146[c]	102	122	146	102	122	146
Alanine	485±69AB	466±100AB	389±50B	499±145AB	522±121AB	549±114AB	554±86AB	598±52A	562±125AB
α-Amino-n-butyric acid	14.7±2.6BCD	13.1±2CD	16.3±2.6BC	25.8±3.9A	16.3±4.9BC	14.8±3.0BCD	11.1±3.8D	10.1±5.8D	18.5±2.2B
Ammonia	399±38AB	488±35A	500±71A	308±84BCD	204±69D	307±113BCD	263±85CD	359±149BC	400±69AB
Arginine	505±31A	468±24A	391±25BC	399±111B	357±50BCD	323±25D	390±21BC	386±51BC	344±25CD
Asparagine	216±21A	174±15B	169±21BC	69.1±23G	106±10EF	133±14DE	97.5±29FG	105±25EF	141±33CD
Aspartic Acid	62.2±15B	48.7±44B	39.5±26B	68.0±47B	52.8±37B	133±25A	145±25A	136±30A	1[illegible]±18A
Citrulline	8.83±1.6A	6.50±1ABC	6.75±0.96AB	4.18±1.7C	5.15±1.4BC	5.78±2.6BC	4.85±2.0BC	5.60±.42BC	6.50±1.0ABC
Cystathionine	9.48±1.7A	8.40±2.0A	7.45±2.5A	6.78±3.7A	23.9±32A	8.88±2.7A	15.0±3.8A	12.5±2.5A	12.1±1.6A
Cysteic acid/phosphoserine	16.7±0.71A	14.7±1.9B	15.0±1.0AB	9.70±1.2D	10.0±1.3CD	10.3±1.8CD	10.6±1.4CD	11.6±1.0C	10.3±1.73CD
Half-cystine	113±2.9A	104±9.0AB	104±11AB	89.2±13B	96.8±11AB	110±14A	113±9.0A	111±6.9A	108±4.4A
Ethanolamine	28.4±4.7A	16.9±4.5A	27.2±8.2A	20.2±14A	23.9±14A	24.4±12A	32.8±18A	29.3±18A	12.0±4.3A
Glutamic acid	152±16ABC	135±12D	147±7.5BCD	134±11D	140±6.7CD	146±9.7BCD	159±7.3AB	165±11A	158±14AB
Glutamine	912±170A	808±97A	715±105A	628±169A	708±198A	707±135A	742±125A	771±151A	694±153A
Glycine	678±52AB	642±44B	613±35B	624±113B	670±131AB	813±132A	726±82AB	694±45AB	726±88AB
Histidine	153±16A	122±33A	101±19A	118±30A	108±40A	134±14A	146±12A	115±35A	141±31A
Hydroxylysine	3.00±0.65A	2.93±0.15A	2.45±0.48A	2.65±0.58A	2.63±0.44A	3.25±0.87A	2.88±.66A	2.80±0.39A	2.98±0.36A
Hydroxyproline	192±25BCD	148±19EF	127±17F	155±37DEF	181±22CDE	194±8.7BC	221±18AB	248±43A	197±36BC
Isoleucine	128±15AB	113±31AB	106±9.8B	131±11AB	135±18AB	143±16A	122±3.8AB	129±22AB	146±29A
Leucine	224±12CD	197±36D	197±18D	221±14CD	211±22CD	261±28AB	262±33AB	244±33BC	285±22A
Lysine	242±11D	330±86CD	479±50B	429±140BC	423±101BC	473±85B	401±74BC	500±80B	753±96A
Methionine	75.1±6.7B	62.9±10C	59.1±6.5C	76.3±7.3B	86.6±5.2A	78.5±4.2AB	80.3±4.8AB	80.4±1.4AB	81.4±10AB
1-Methylhistidine	21.1±2.0A	14.0±2.7A	19.7±2.3A	16.4±7.6A	14.0±2.3A	14.4±8.5A	10.6±5.6A	49.3±41A	17.4±3.3A
3-Methylhistidine	16.9±1.7DE	15.8±3.4E	22.9±1.5A	21.0±2.8AB	18.1±1.6BCDE	19.6±.56BCD	15.7±1.9E	17.7±.93CDE	19.9±2.2BC
Ornithine	74.0±8ABC	93.6±21A	69.1±5.7BC	62.8±19BC	53.3±14C	78.8±12AB	81.4±13AB	71.6±12ABC	75.5±16ABC
Phenylalanine	226±25AB	208±23B	245±34A	108±43CD	101±25D	111±28CD	127±21CD	130±10CD	137±11C
Proline	547±56A	492±112A	428±20A	498±138A	518±49A	578±101A	521±89A	589±60A	[illegible]20±100A
Serine	586±53ABC	550±30BC	514±49C	642±44AB	614±84AB	619±69AB	619±56AB	653±19A	587±74ABC
Taurine	284±5.9A	219±54AB	270±47A	181±84BC	146±47C	150±18BC	130±30C	150±30BC	149±33C
Threonine	401±61DE	403±98DE	356±67E	477±152CDE	515±113BCD	570±112ABC	708±51A	635±90AB	701±97A
Tryptophan	18.6±27A	5.00±10A	3.25±6.5A	0.00±0.0A	0.0±0.0	0.0±0.0A	0.00±0.0A	0.0±0.0A	10.0±0.0A
Tyrosine	273±37A	237±14B	206±23BC	169±13E	175±27CDE	193±21CDE	170±41DE	174±13CDE	204±13BCD
Valine	281±23ABC	243±42BC	239±21C	284±64ABC	298±40ABC	315±33AB	290±43ABC	327±29A	311±56ABC

[a] Values (means ± standard deviations) for FAA or NPS followed by the same letter are not significantly different at $P \leq 0.05$
[b] Pair fed to the feed intake of the corresponding aflatoxin group.
[c] Percentage of the NRC dietary requirement (1977) of lysine (basal ration provides 102%).
[d] ND = Not detectable at levels less than ca 0.1 nmol/ml: α-aminoadipic acid, anserine, γ-aminobutyric acid, β-aminoisobutyric acid, carnosine, glucosamine, glycerophosphoethanolamine, homocitrulline, homocystine, methionine sulfone or sulfoxide, phosphoethanolamine.

App. 24a. Major and interactive effects produced by graded levels of dietary lysine on concentrations of free-amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

FAA or NPS	Free-amino acid or ninhydrin positive substances (nmol/ml of plasma)[a]								
	Effect of feeding			Effect of administration			ANOVA [F (Pr> F)][e]		
	Aflatoxin[b]	Restricted[c]	Ad libitum	102	122	146[d]	Feed	Admin	Feed x Admin.
Alanine	447B	523AB	571A	-A	-A-	-A	4.67(0.018)	0.24(0.79)	0.63(0.65)
α-amino-n-butyric acid	14.7B	19.0A	14.7B	17.2A	13.2B	16.5A	8.12(0.0017)	4.34(0.023)	6.98(0.0005)
Ammonia	463A	273C	341B	324A	350A	402A	15.0(0.0001)	2.60(0.092)	1.82(0.15)
Arginine	455A	360B	373B	431A	404A	353B	21.2(0.0001)	12.8(0.0001)	0.97(0.44)
Asparagine	50.1C	84.4B	146A	91.8AB	79.1B	109A	28.4(0.0001)	2.78(0.080)	2.71(0.051)
Asparatic acid	186A	102B	114B	127B	128AB	147A	49.7(0.0001)	3.15(0.059)	7.46(0.0003)
Citrulline	7.36A	5.00B	5.65B	-A	-A	-A	7.65(0.0023)	0.41(0.67)	3.28(0.087)
Cystathionine	-A	-A	-A	-A	-A	-A	0.74(0.49)	0.84(0.44)	1.08(0.38)
Cysteic acid/phosphoserine	15.4A	10.0B	10.8B	-A	-A	-A	58.9(0.0001)	0.35(0.071)	1.78(0.16)
Half-cystine	107A	98.6B	111A	-A	-A	-A	4.75(0.017)	0.31(0.73)	2.92(0.040)
Ethanolamine	-A	-A	-A	-A	-A	-A	0.08(0.93)	0.77(0.48)	1.99(0.13)
Glutamic acid	145B	140B	161A	-A	-A	-A	11.3(0.0003)	0.42(0.66)	1.94(0.13)
Glutamine	-A	-A	-A	-A	-A	-A	2.37(0.11)	0.57(0.57)	0.94(0.46)
Glycine	-A	-A	-A	-A	-A	-A	2.20(0.13)	1.06(0.36)	2.33(0.082)
Histidine	-A	-A	-A	-A	-A	-A	0.77(0.47)	2.30(0.12)	1.83(0.15)
Hydroxylysine	-A	-A	-A	-A	-A	-A	0.09(0.92)	0.12(0.89)	1.44(0.25)
Hydroxyproline	156C	177B	222A	-A	-A	-A	18.4(0.0001)	1.81(0.18)	4.91(0.0042)
Isoleucine	116B	136A	132A	-A	-A	-A	3.39(0.032)	0.30(0.74)	1.52(0.23)
Leucine	206C	231B	263A	236AB	217B	247A	15.5(0.0001)	4.37(0.025)	2.10(0.11)
Lysine	351C	442B	552A	358B	418B	569A	16.0(0.0001)	18.7(0.0001)	3.31(0.025)
Methionine	65.7B	80.4A	80.7A	-A	-A	-A	19.2(0.0001)	1.39([illegible]7)	3.67)0.016)
1-Methylhistidine	-A	-A	-A	-A	-A	-A	1.83(0.18)	1.67(0.21)	3.52(0.019)
3-Methylhistidine	-A	-A	-A	17.9B	17.2B	20.8A	2.38(0.11)	10.8(0.0004)	5.04(0.0036)
Ornithine	78.9A	65.0B	76.1AB	-A	-A	-A	3.23(0.055)	0.05(0.95)	3.50(0.02)
Phenylalanine	226A	107C	131B	-A	-A	-A	68.8(0.001)	1.37(0.27)	0.[illegible]1(0.80)
Proline	-A	-A	-A	-A	-A	-A	1.27(0.30)	0.23(0.79)	1.65(0.19)
Serine	550B	625A	620A	-A	-A	-A	6.59(0.0047)	1.87(0.17)	0.73(0.58)
Taurine	258A	159B	143B	-A	-A	-A	20.1(0.0001)	0.95(0.40)	0.96(0.45)
Threonine	386C	521B	681A	-A	-A	-A	27.3(0.0001)	0.19(0.83)	0.86(0.50)
Tyrosine	239A	179B	182B	-A	-A	-A	22.2(0.0001)	0.42(0.66)	5.22(0.0030)
Valine	255B	299A	310A	-A	-A	-A	5.97(0.0071)	0.04(0.96)	1.30(0.30)
Tryptophan	9.94A	0.00B	0.00B	-A	-A	-A	3.30(0.05)	0.97(0.39)	0.97(0.44)

[a]Means for FAA or NPS within a major effect followed by the same letter are not significantly different at P<0.05.

[b]Dietary aflatoxin = 2.5μg per g of diet.

[c]Pair-fed to the feed intake of the corresponding aflatoxin group.

[d]Percentage of the NRC dietary requirement (1977) of lysine (basal ration provided 102%).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x Admin = test for interaction.

App. 25. Effect of graded levels of lysine and arginine on the concentrations of plasma free amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

	Free-amino acid or ninhydrin positive substance (nmol/ml of plasma)[a]											
	Aflatoxin (2.5 µg/g diet)				Restricted Feeding[b]				Ad libitum controls			
	94 Arg[c]	122 Arg			94 Arg	122 Arg			94 Arg	122 Arg		
FAA or NPS	102 Lys	102 Lys[c]	122 Lys[c]	146 Lys[c]	102 Lys	102 Lys	122 Lys	146 Lys	102 Lys	102 Lys	122 Lys	146 Lys
Alanine	510±89BC	554±16ABC	455±42C	558±68ABC	534±37ABC	656±95A	626±94AB	535±94ABC	467±81C	461±109C	548±29ABC	502±84BC
α-Amino-n-butyric Acid	24.9±6.7B-F	13.9±1.2F	18.9±6.0C-F	17.5±4.3DEF	33.0±8.5AB	29.9±7.4ABC	29.4±12A-D	15.4±4.7F	37.1±12A	28.1±15A-E	15.3±1.4F	16.1±0.8EF
Ammonia	327±31A	256±24B	249±13BC	257±31B	215±12BCD	207±71CDE	226±47BCD	254±51B	171±21E	247±17BC	205±11DE	248±44BC
Arginine	501±96AB	547±59A	433±29BC	443±55ABC	345±45CD	448±129ABC	445±129ABC	457±62AB	316±37D	283±42D	455±57AB	314±53D
Asparagine	51.4±7.1BC	81.3±6.1A	66.4±21AB	74.4±13A	34.4±3.4E	55.4±17BC	49.2±13CDE	54.6±11BC	36.9±13DE	44.2±2.1CDE	49.7±3.4CDE	52.6±8.6BCD
Aspartic acid	151±43BC	191±20A	145±9.4CD	140±8.3CDE	114±18DE	145±20CD	150±27BCD	200±41A	106±10E	122±20CDE	212±30A	186±38AB
Citrulline	3.98±0.1CD	5.75±1.5AB	4.55±0.5BCD	4.00±1.2CD	3.75±0.6CD	4.25±1.5BCD	5.38±1.0ABC	6.45±1.0A	6.40±2.2A	2.95±0.1D	5.88±0.3AB	4.53±1.3BCD
Cystathionine	11.6±23BC	8.40±0.8BC	7.73±1.2C	7.88±0.9C	12.9±55B	7.13±1.4C	11.1±3.8BC	10.2±3.6BC	10.9±6.5A	11.2±2.0BC	11.6±0.9BC	10.5±1.2BC
Cysteic acid	14.8±2.7A	9.65±1.6BC	105±1.6B	10.0±1.1BC	8.88±0.3BCD	6.60±1.9D	8.10±2.6BCD	7.45±2.9CD	8.80±1.0BCD	7.83±1.7CD	7.43±2.0CD	8.85±0.6BCD
Half Cystine	128±17A	111±13ABC	101±17BCD	95.3±7.7CD	88.7±16D	83.5±13D	99.1±7.8BCD	87.1±4.5D	126±13A	87.9±16D	115±8.4AB	96.9±12BCD
Ethanolamine	11.7±4.8CDE	20.0±2.0AB	15.6±2.9B-E	17.0±3.9BCD	10.0±5.3DE	25.6±12A	18.7±9.1ABC	13.3±4.3B-E	7.45±0.6E	11.8±3.3CDE	13.5±4.9B-E	14.8±2.5B-E
Glutamic acid	134±61BCD	161±14A	145±11AB	142±18ABC	102±11F	126±18B-E	123±21CDE	121±14DEF	115±16DEF	114±7.8EF	126±7.6B-E	124±8.4CDE
Glutamine	892±85ABC	939±37A	881±57A-D	925±71AB	782±44D	806±76CD	864±80A-D	665±117E	828±106BCD	636±44E	822±32CD	670±79E
Glycine	583±64AB	649±55AB	554±52AB	650±24AB	545±40B	576±106AB	554±79AB	667±122AB	672±40A	570±30AB	649±47AB	643±73AB
Histidine	143±13AB	160±13A	144±9.6AB	152±12A	87.2±7.4D	118±25C	109±24C	124±20BC	117±12C	112±8.6C	156±16A	121±17C
Hydroxylysine	2.48±1.0A	4.88±1.8A	5.50±3.2A	4.33±3.4A	3.28±0.6A	6.58±1.2A	5.70±2.8A	4.73±3.0A	5.80±2.7A	4.18±1.8A	5.50±1.5A	3.65±1.8A
Hydroxyproline	122±19B-E	93.1±11E	95.6±4.8DE	104±24CDE	201±45A	110±6.0DE	116±8.7B-E	124±26BCD	213±38A	131±13BC	138±11B	124±8.5BCD
Isoleucine	122±23BC	185±12A	156±18ABC	142±22BC	124±31BC	155±37ABD	140±21BC	161±39AB	134±14BC	117±11C	139±21BC	161±24AB
Leucine	234±36B-E	326±24A	282±28AB	263±24BCD	208±40E	272±60BC	245±26B-E	277±28BC	233±20CDE	215±15DE	252±34B-E	277±33BC
Lysine	425±58E	437±90E	487±96CDE	705±59ABC	462±138DE	873±276A	726±116ABC	717±166ABC	600±145B-E	843±254A	691±132A-D	792±203AB
Methionine	77.2±12A	71.4±8.6A	59.1±10A	62.8±11A	69.2±12A	80.1±22A	77.1±9.4A	64.6±13A	80.2±5.1A	61.0±12A	68.2±10A	64.1±13A
1-Methylhistidine	11.0±2.1CD	13.9±1.1BC	20.7±11A	18.1±4.7AB	8.68±4.0CD	10.4±2.2CD	5.25±2.5D	10.1±1.0CD	11.7±3.5C	14.5±2.4ABC	11.2±1.8CD	13.3±2.6BC
3-Methylhistidine	18.5±3.2BCD	12.4±2.6E	15.0±2.9CDE	16.5±3.3BCD	24.6±3.1A	18.7±2.8BC	14.6±2.2DE	14.7±2.9DE	20.1±4.0B	17.2±2.9BCD	18.5±1.7BCD	16.1±1.1BCD
Ornithine	93.2±9.7CDE	237±29A	146±37BC	156±16BC	29.0±2.7E	52.4±19E	58.3±36DE	188±14AB	67.4±30DE	89.5±24CDE	173±29AB	127±51BCD
Phenylalanine	194±23B	243±34A	165±11C	156±17CD	107±15F	125±21EF	123±14EF	119±10EF	119±7.0EF	115±6.2EF	135±13DE	134±24DE
Proline	411±54CDE	586±19A	456±37BCD	471±31BCD	279±22F	340±84EF	346±80EF	540±159AB	380±64DE	331±15EF	518±43AB	409±99ABC
Serine	526±42G	682±56DE	565±75FG	625±106DEF	852±55B	999±81A	970±46A	807±110BC	507±116EFG	674±30DE	659±130EF	713±60CD
Taurine	436±87AB	357±83AB	230±49CDE	244±56CD	166±67DEF	299±128B	295±86BC	118±22F	185±52DEF	180±46DEF	146±35EF	142±26EF
Threonine	482±85EF	536±37CDE	468±79EF	418±61F	509±58DEF	561±73B-E	608±62BCD	639±127BC	521±39DEF	675±114AB	767±60A	662±127AB
Tyrosine	202±24AB	222±14A	180±10BC	187±9.4BC	128±17E	178±30BC	173±26C	127±17E	143±16DE	161±15CD	133±18E	139±21DE
Tryptophan	71.9±7.1BC	92.6±5.1A	86.4±5.0A	75.0±7.5B	64.5±4.6C	90.4±7.0A	88.7±8.2A	88.4±5.9A	68.7±9.6BC	60.3±7.7BC	93.8±4.4A	86.7±8.8A
Valine	283±32BCD	360±9.4A	306±22ABC	276±28BCD	235±32D	268±66BCD	266±21BCD	319±93AB	305±29ABC	242±22CD	295±37A-D	324±46AB

[a]Values (means ± standard deviations) for FAA or NPS followed by the same letter are not significantly different at $P \leq 0.05$.
Not detectable at levels less than ca 0.1nmol/ml: α-aminoadipic acid, anserine, γ-aminobutyric acid, β-aminoisobutyric acid, carnosine, glucosamine, glycerophosphoethanolamine, homicitrulline, homocystine, methionine sulfone or sulfoxide, phosphoethanolamine, sarcosine.
[b]Pair fed to the feed intake of the corresponding aflatoxin group.
[c]Percentage of the NRC dietary requirement (1977) of lysine or arginine (basal ration provides 94% and 102%, respectively).

App.23a. Major and interactive effects produced by graded levels of lysine and arginine on the concentrations of plasma free amino acids (FAA) and other ninhydrin positive substances (NPS) in plasma of chicks with aflatoxicosis.

Free-amino acid or ninhydrin positive substances (nmol/ml of plasma)[a]

FAA or NPS	Effect of feeding			Effect of administration[d]				ANOVA (F[Pr>F])[e]		
	Aflatoxin[b]	Restricted[c]	Ad libitum	94 Arg+102 Lys	122 Arg+102 Lys	122 Arg+122 Lys	122 Arg+146 Lys	Feed	Admin	Feed x Admin
Alanine	519B	589A	499B	-A	-A	-A	-A	6.13(0.0051)	1.26(0.30)	19.4(0.10)
α-Amino-n-butyric acid	18.8B	26.9A	24.1AB	31.7A	23.9B	21.28C	16.3C	4.28(0.022)	7.72(0.0004)	2.12(0.075)
Ammonia	272A	223B	218B	-A	-A	-A	-A	15.4(0.0001)	1.56(0.22)	612(0.0002)
Arginine	481A	424B	342C	-A	-A	-A	-A	14.4(0.0001)	1.35(0.27)	3.59(0.0068)
Asparagine	-A	-A	-A	124C	153B	169AB	175A	0.15(0.86)	9.03(0.0001)	7.93(0.0001)
Aspartic acid	68.4A	48.4B	45.8B	40.9B	60.3A	55.1A	60.5A	19.0(0.0001)	7.94(0.0003)	0.83(0.56)
Citrulline	-A	-A	-A	-A	-A	-A	-A	0.62(0.54)	1.61(0.20)	6.57(0.0001)
Cystathionine	8.91B	10.3B	13.0A	14.5A	8.90B	10.1B	9.51B	7.24(0.0023)	7.95(0.0003)	1.15(0.36)
Cysteic acid/phosphoserine	11.2A	7.76B	8.23B	10.8A	8.03B	8.68B	8.78B	16.9(0.0001)	5.19(0.0001)	1.61(0.17)
Half cystine	109A	89.6B	106A	114A	94.1B	105B	93.1B	10.6(0.0002)	7.17(0.0007)	3.04(0.017)
Ethanolamine	16.1A	16.9A	11.9B	9.7B	19.1A	15.9A	15.0	4.16(0.024)	6.57(0.0012)	1.65(0.16)
Glutamic acid	145A	118B	119B	117B	133A	131A	129AB	20.5(0.0001)	3.55(0.024)	1.29(0.28)
Glutamine	909A	779B	734B	834A	794AB	856A	753B	23.5(0.0001)	4.58(0.0081)	4.31(0.0022)
Glycine	-A	-A	-A	600AB	599AB	586B	653A	2.05(0.14)	2.37(0.087)	2.06(0.083)
Histidine	150A	109C	126B	116B	130A	137A	132A	26.4(0.0001)	3.88(0.17)	3.88(0.0044)
Hydroxylysine	-A	-A	-A	-A	-A	-A	-A	0.50(0.61)	1.58(0.21)	1.14(0.36)
Hydroxyproline	104B	178A	151A	179A	111B	117B	117B	20.6(0.0001)	25.8(0.0001)	2.74(0.027)
Isoleucine	-A	-A	-A	127B	152A	145AB	155A	1.26(0.30)	3.22(0.034)	2.80(0.025)
Leucine	274A	250B	244B	225B	271A	260A	272A	4.40(0.020)	5.64(0.0028)	3.30(0.011)
Lysine	513B	694A	732A	496B	718A	634A	738A	8.60(0.0009)	5.72(0.0026)	1.70(0.15)
Methionine	-A	-A	-A	-A	-A	-A	-A	0.83(0.45)	1.94(0.14)	1.58(0.18)
1-Methylhistidine	15.9A	8.62C	12.6B	-A	-A	-A	-A	12.5(0.0001)	1.41(0.26)	2.38(0.049)
3-Methylhistidine	15.6B	18.2A	18.0A	21.0A	16.1B	16.0B	15.8B	4.20(0.023)	9.86(0.0001)	3.17(0.014)
Ornithine	158A	81.8B	114.B	63.2B	126A	126A	157A	9.43(0.0005)	7.52(0.0005)	4.98(0.0008)
Phenylalanine	190A	119B	126B	140B	161A	141B	137B	75.4(0.0001)	4.68(0.0073)	8.24(0.0001)
Proline	481A	376C	430B	357C	419B	440B	500A	8.65(0.0009	8.31(0.0003)	6.24(0.0001)
Serine	600C	907A	658B	655C	785A	732AB	715B	80.4(0.0001)	6.47(0.0013)	2.76(0.026)
Taurine	317A	219B	163C	262AB	279A	223B	168C	21.1(0.0001)	6.37(0.0014)	4.87(0.001)
Threonine	476A	579B	656A	504A	590A	615A	573A	19.4(0.0001)	3.99(0.015)	2.69(0.029)
Tryptophan	-A	-A	-A	68.3C	83.7B	89.6A	83.2B	1.15(0.33)	20.5(0.0001)	6.84(0.0001)
Tyrosine	198A	151B	144B	158B	187A	162B	151B	37.5(0.0001)	8.20(0.0003)	2.80(0.024)
Valine	304A	272B	291AB	-A	-A	-A	-A	2.62(0.086)	1.13(0.35)	3.72(0.0056)

[a]Means for a FAA or NPS within a major effect followed by the same letter are not significantly different at $P \leq 0.05$.
[b]Dietary aflatoxin = 2.5 μg per g of diet.
[c]Pair-fed to the feed intake of the corresponding aflatoxin group.
[d]Percentage of the NRC dietary requirement (1977) of lysine or arginine (basal ration provided 102% and 94% respectively).

[e]Admin = major effect of administration. Feed = major effect of feeding. Feed x admin = test for interaction.