Director's Digest
FATS AND PROTEINS RESEARCH FOUNDATION, INC.

GARY G. PEARL D.V.M.
Director Technical Services

R.R. #2 Box 298
Bloomington, Illinois 61704
Telephone: 309-829-7744 FAX: 309-829-5147

EFFECTS OF BIOGENIC AMINES ON BROILER PERFORMANCE

#296

MELANIE L. FRIDAY and JEFFRE D. FIRMAN[1]
Department of Animal Sciences, College of Agriculture, 116 Animal Science Research Center, University of Missouri, Columbia, MO 65211
Phone: (573) 882-9427
FAX: (573) 882-6640
E-mail: firmanj@missouri.edu

ALEX J. BERMUDEZ
Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, P.O. Box 6023, University of Missouri, Columbia, MO 65211

Primary Audience: Broiler Integrators, Veterinarians, Feed Manufacturers

SUMMARY

This study was designed to determine whether the biogenic amines phenylethylamine, putrescine, cadaverine, and histamine, alone or in combination, if added at levels above those commonly found in complete mixed rations containing animal by-product meals, would cause lesions or reduce performance in broilers. The levels of biogenic amines used were double those that have been previously reported to cause problems for broilers. Data collected on performance, gross pathology, and histopathology at 3 and 6 wk indicated no significant differences in production parameters among amine treatments. No significant gross lesions or histopathologic changes were evident. This study suggests that the four studied biogenic amines are of little concern to the broiler industry when fed in the amounts utilized.

Key words: Biogenic amine, broiler, cadaverine, histamine, phenylethylamine, putrescine
1999 J. Appl. Poultry Res. 8:408–413

DESCRIPTION OF PROBLEM

Animal by-product meals from meat rendering are a major component of many poultry feeds. By-product feeds are used to meet protein (amino acid) requirements in corn-soybean-type diets in a cost-efficient manner, in some cases providing a growth response.

Biogenic amines are organic bases of low molecular weight that exhibit biological activity and are usually produced by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones [1]. The use of animal by-product meals in poultry feeds allows the opportunity for the presence of biogenic amines. The toxicity of high levels of biogenic amines resulting from the presence of spoiled animal by-products has been suggested as a causative factor in reduced broiler performance and intestinal lesions [2].

Johnson and Pinedo [3] reported on gizzard erosions and ulceration in Peru broilers in 1971. Harry and Tucker [4, 5] associated the incidence of suppressed weight gain and gizzard erosion with histamine presence. Keirs and Bennet [2] associated increased biogenic amine levels with loss of feed efficiency in broiler production. These affected

broilers exhibited a syndrome of passage of undigested feed, stained and dirty vent feathers, and a lesion commonly seen in the proximal section of the duodenal loop consisting of a layer of necrosed intestinal epithelial cells. However, no controlled studies were performed to determine whether the biogenic amines in fact caused this syndrome.

Bermudez and Firman [6] studied cadaverine, histamine, phenylethylamine, and putrescine in diets at the highest concentrations likely to occur in diets fed in the United States. No deleterious production effects or lesions were found. The current study was designed to determine whether biogenic amines, at levels double those found in the previous study, would cause reduced performance, clinical signs of disease, or lesions in broilers.

Materials and Methods

EXPERIMENTAL DESIGN

The study consisted of 12 treatments with four pen replicates per treatment and 30 broilers per pen. Treatments were randomized within blocks of 12 pens. The experiment was a randomized block design with a two-way factorial arrangement of treatments. One main effect was a corn-soybean diet (CS) vs. a corn-soybean diet with 10% added by-product meal (BP). The other main effect was the addition of no amine (control) or the addition of phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), or a combination (COMB) of all the above amines (Sigma Chemical Co., Inc., St. Louis, MO). Diets were formulated to be isocaloric and isonitrogenous. Levels of biogenic amine fed were double those used in our previously reported research [6] and should represent levels exceeding those found in practical diets. On the basis of commercial ration formulation standards, it was assumed that no more than 10% animal by-product meal would be commonly used in a ration. The biogenic amines, PHE, PUT, CAD, HIS, were added at 9.6 mg/kg, 98 mg/kg, 214 mg/kg, and 262 mg/kg, respectively. These rations were fed to the broilers from hatch to 6 wk of age, at which time the study was terminated. The COMB treatment contained all of the amines at these levels, while the control contained no added amine.

Cobb-Ross cross broilers from a commercial hatchery were used in the study. Birds were maintained on litter floors under commercial-type conditions in a curtain-sided building. Broilers were weighed and feed intake measured at 3 and 6 wk of age. Data collected were used to calculate body weight gain and the feed:gain ratio. Mortality was monitored on a daily basis. All nutrients met or exceeded National Research Council recommendations [7].

DIETS

All feedstuffs used in the experiment were assayed for biogenic amine content [8]. Briefly, the procedure used is based on the separation of dansyl derivatives of the amines using reversed-phase liquid chromatography with gradient elution followed by ultraviolet detection at 254 nm. Analysis confirmed that all feed ingredients used in the experimental rations were below detection levels with the exception of meat and bone meal. Since by-product meal was 10% of the diet, the highest level of naturally occurring biogenic amine in experimental rations was 0.3 mg/kg. Rations are detailed in Table 1. Both animal proteins used could be considered high-quality products. A variety of suspect animal-derived meals were tested previously in an attempt to find a meal with high amine content with no success.

GROSS PATHOLOGY AND HISTOPATHOLOGY

Post-mortem examinations were performed on 16 broiler chickens from each treatment group (4 chicks per replicate) at 3 and 6 wk of age. Chickens were euthanized with carbon dioxide followed by cervical dislocation. A general gross examination of each bird was performed and the mucosal surfaces of the proventriculus, ventriculus, duodenum, and jejunum were examined for gross pathology. The proventriculus was excised and opened, the contents removed, and the proventricular weight determined. Samples of proventriculus, ventriculus, duodenum, pancreas, and jejunum were collected from five chickens per treatment and fixed in 10% neutral buffered formalin. Fixed tissue samples were trimmed, embedded in paraffin, sectioned at 4 μm and slides stained with hematoxylin and

TABLE 1. Diet composition

INGREDIENT	STARTER CONTROL	STARTER BY-PRODUCT	GROWER CONTROL	GROWER BY-PRODUCT
Corn	53.683	60.889	63.047	69.977
Soybean meal 48%	38.230	25.106	30.57	17.48
Fish, Menhaden	0	5.00	0	5.00
Meat and bone meal	0	5.00	0	5.00
Lard	4.200	2.60	3.00	1.50
Salt	0.300	0.300	0.300	0.300
Dicalcium phosphate	2.709	0.243	1.49	0.029
Met-MHA	0.204	0.177	0.075	0.053
Limestone	1.157	0.158	1.08	0.217
Lysine•HCl	0.121	0.120	0.056	0.068
Mineral premix[A]	0.100	0.100	0.100	0.100
Coban	0.075	0.075	0.075	0.075
Vitamin premix[B]	0.075	0.075	0.075	0.075
Choline chloride	0.053	0.063	0.029	0.039
Selenium premix[A]	0.030	0.030	0.030	0.030
Bacitracin	0.050	0.050	0.050	0.050
Copper sulfate	0.013	0.013	0.013	0.013
CALCULATED ANALYSIS				
Protein, %	23.0	23.0	20.0	20.0
ME, kcal/kg	3200	3200	3200	3200
Lysine, %	1.36	1.35	1.11	1.11
TSAA, %	0.90	0.90	0.72	0.72
Calcium, %	1.00	1.00	0.90	0.90
Available phosphorus, %	0.45	0.45	0.45	0.45

[A]Mineral mixes provided the following per kg of diet: manganese, 110 mg; zinc, 110 mg; iron, 60 mg; iodine, 2 mg; magnesium, 27 mg; selenium, 0.18 mg; copper, 44 mg.

[B]Vitamin premix supplied the following amounts per kg of diet: vitamin A as vitamin A acetate, 7700 IU; vitamin D_3 as cholecalciferol, 2750 IU; vitamin E, as dl-α-tocopherol acetate, 11 IU; niacin, 44 mg; d-pantothenic acid, 13.2 mg; riboflavin, 5.5 mg; vitamin B_6, 2.2 mg; menadione, 1.65 mg; folic acid, 1.1 mg; thiamine, 1.1 mg; biotin, 0.11 mg; vitamin B_{12}, 8.8 μg.

eosin stain. Tissue sections from all treatment groups were examined microscopically.

All data were analyzed by factorial analysis of variance with the pen considered the experimental unit [9]. The significance levels were maintained at 0.05 unless otherwise noted.

Results and Discussion

Results of the performance data are presented in Table 2. At 3 wk of age the addition of by-products improved growth rate slightly at the P = .06 level.

Additions of animal-derived proteins have been shown to provide small growth responses in many instances. This improvement may be due to a number of factors, including better balance of amino acids, incorrectly calculated energy values for meat meals, high digestibility of fish meals, and a reduction in antinutritional factors such as the oligosaccharides concomitant with the reduction in soybean meal [10, 11].

Feed efficiency was not significantly affected. No effects were noted from addition of amines to the diets. At 6 wk of age, no differences were noted in any of the performance parameters measured. In our previous study [6] no consistent effects of added amines were

TABLE 2. Effects of by-product additions or biogenic amine additions on weight gain and feed:gain ratio of broilers

DIET[A]	CONTROL	HIS	PUT	CAD	PHE	COMB	MEAN
3 WK WEIGHT (g)							
CS	699	755	729	721	719	687	715
BP	761	747	764	784	772	764	765
MEAN	730	751	746	753	745	726	
POOLED SEM	22.59						
3 WK FEED:GAIN							
CS	1.46	1.41	1.46	1.47	1.46	1.47	1.46
BP	1.40	1.40	1.40	1.38	1.37	1.36	1.38
MEAN	1.43	1.41	1.43	1.42	1.42	1.41	
POOLED SEM	0.030						
6 WK WEIGHT (g)							
CS	1994	2060	2083	2092	1978	2009	2034
BP	2045	2059	2068	2111	2030	2045	2060
MEAN	2019	2059	2075	2010	2004	2027	
POOLED SEM	35.3						
6 WK FEED:GAIN							
CS	1.73	1.72	1.74	1.74	1.74	1.72	1.73
BP	1.72	1.73	1.72	1.70	1.72	1.70	1.71
MEAN	1.72	1.73	1.73	1.72	1.73	1.71	
POOLED SEM	0.010						

ANOVA EFFECTS[B]				
COMPARISON	3 WK WEIGHT	3 WK FEED:GAIN	6 WK WEIGHT	6 WK FEED:GAIN
Diet	P = .06	NS	NS	NS
Amine	NS	NS	NS	NS
Diet × Amine	NS	NS	NS	NS

[A]CS = Corn-soybean meal diet; BP = Diet with by-products added, controls with no amines added; HIS = histidine added at 262 mg/kg; PUT = putrescine added at 98 mg/kg; CAD = cadaverine added at 214 mg/kg; PHE = phenylalanine added at 9.6 mg/kg; COMB = all above amines at above levels simultaneously.

[B]NS = Not significant.

noted, although a similar by-product effect was found.

No consistent gross pathology was evident in any of the birds examined. No gross pathology of the mucosal surface of the proventriculus, ventriculus, duodenum, and jejunum was noted. No treatment-related histopathology changes were evident in sections of proventriculus, ventriculus, duodenum, pancreas, and jejunum in the 12 treatments examined.

Histamine has been implicated as a cause of gizzard ulceration in previous research [3, 4, 12], although the levels used were substantially higher than in this study and much higher than those that should be found in practical broiler diets. No lesions of this type were found in this study or our previous research [6]. The levels of histamine fed in this study were about 20% of the levels at which ulceration began to occur in previously reported research [5]. The levels fed in this study should be twice as much as would be the highest level found in a practical diet in the U.S.

Research studying the effects of putrescine additions to broiler and Japanese quail diets indicated that performance was not affected by the addition [12, 13]. When fed in combination, PUT, CAD, and HIS have been found to cause proventricular enlargement [14]. However, the levels fed in that study were

TABLE 3. Average proventricular weights for each treatment

DIET[A]	CONTROL	HIS	PUT	CAD	PHE	COMB	MEAN
3 WK WEIGHT (g)							
CS	4.456	4.588	4.800	4.519	4.363	4.450	4.529
BP	4.100	4.525	4.300	4.288	4.263	4.338	4.302
MEAN	4.278	4.556	4.550	4.404	4.313	4.394	
6 WK WEIGHT (g)							
CS	9.1438	9.4	10.619	8.713	8.95	0.019	9.307
BP	8.763	8.794	8.631	8.606	8.744	8.556	8.682
MEAN	8.9534	9.097	9.625	8.6595	8.847	8.7875	

ANOVA EFFECTS[B]		
COMPARISON	3 WK WEIGHT	6 WK WEIGHT
Diet	*	*
Amine	NS	*
Diet × Amine	NS	*

[A]CS = corn-soybean meal diet; BP = diet with by-products added, controls with no amines added; HIS = histidine added at 262 mg/kg; PUT = putrescine added at 98 mg/kg; CAD = cadaverine added at 214 mg/kg; PHE = phenylalanine added at 9.6 mg/kg; COMB = all above amines at above levels simultaneously.

[B]NS = Not significant; *Significant at $P < .05$.

significantly higher than used in this trial. Table 3 presents the data collected on proventricular weights in the present study. The enlargement of the proventriculus was very mild, and although statistically significant due to diet at 3 wk and interactive effects at 6 wk, was not considered physiologically relevant.

The levels of amines fed in the current study were double the highest levels that should be found in a practical feeding situation, based on the levels of amines found in previous feed surveys [2]. In a previous screening of a number of feeds by our lab, no high levels of naturally occurring amines were found. The results of the present study support our previous research and indicate that there are no deleterious effects from feeding PHE, PUT, CAD, and HIS to broilers at levels double those used in previous research [6].

Conclusions and Applications

1. Based on the current study and previously reported work [6], there appears to be little likelihood of reductions in broiler performance due to the biogenic amines at levels commonly found in practical diets.
2. The feeding of a product that has been through a spoilage process has inherent risks from a number of potentially toxic products, but it does not appear that the biogenic amines should be of concern in and of themselves.
3. The high levels of amines found in some feedstuffs may be indicative of spoilage; such materials thus should be fed with caution.

References and Notes

1. **Georgiaras, I., G.A. Dykes, and A. von Holy,** 1995. Biogenic amine formation by poultry-associated spoilage and pathogenic bacteria. Lett. Appl. Microbiol. 21:164–166.

2. **Keirs, R.W. and L. Bennet,** 1993. Broiler performance loss associated with biogenic amines. Pages 31–34 in: Proc. Maryland Nutr. Conf., Baltimore, MD.

3. **Johnson, D.C. and C. Pinedo D.,** 1971. Gizzard erosion and ulceration in Peru broilers. Avian Dis. 15:835–837.

4. **Harry, E.G. and J.F. Tucker,** 1976. The effect of orally administered histamine on the weight gain and development of gizzard lesions in chicks. Vet. Rec. 99:206–207.

5. **Harry, E.G., J.F. Tucker, and A.P. Laursen-Jones,** 1975. The role of histamine and fish meal in the incidence of gizzard erosion and pro-ventricular abnormalities in the fowl. Br. Poultry Sci. 16:69–78.

6. **Bermudez, A.J. and J.D. Firman,** 1998. Effects of biogenic amines in broiler chickens. Avian Dis. 42:199–203.

7. **National Research Council,** 1994. Nutrient Requirements of Domestic Animals. Nutrient Requirements of Poultry. 9th Rev. Edition. Natl. Acad. Press, Washington, DC.

8. **Moret, S., R. Bortolomeazzi, and G. Lercker,** 1992. Improvement of extraction procedure for biogenic amines in foods and their high-performance liquid chromatographic determination. J. Chromatogr. 591:175–180.

9. **SAS Institute, Inc.,** 1995. JMP User's Guide. SAS Institute, Inc., Cary, NC.

10. **Boling, S.D. and J.D. Firman,** 1997. Rendered by-products as soybean meal replacement in turkey rations. J. Appl. Poultry Res. 6:210–215.

11. **Leeson, S. and J.D. Summers,** 1991. Commercial Poultry Nutrition. University Books, Guelph, ON, Canada.

12. **Colnago, G.L. and L.S. Jensen,** 1992. Putrescine effects on performance of male broiler chicks fed low-protein diets supplemented with essential amino acids. Poultry Sci. 71:211–214.

13. **Blonz, E.R. and H.S. Olcott,** 1978. Effect of histamine, putrescine, and of canned spoiled tuna on growth of young Japanese quail. J. Food Sci. 43:1390–1391.

14. **Stuart, B.P., R.J. Cole, E.R. Waller, and R.E. Vesonder,** 1986. Proventricular hyperplasia (malabsorption syndrome) in broiler chickens. J. Environ. Pathol. Toxicol. Oncol. 6:369–385.