The Impact of Yeast Culture Residue on the Suppression of Dietary Aflatoxin on the Performance of Broiler Breeder Hens

V. G. Stanley,*,† M. Winsman,* C. Dunkley,* and T. Ogunleye,* M. Daley,* W. F. Krueger,† A. E. Sefton,‡ and A. Hinton, Jr.§

*Prairie View A&M University, Prairie View, Texas 77446; †Poultry Science Department, Texas A&M University, College Station, Texas 77845; ‡Alltech, Guelph, Canada; and §Poultry Processing and Meat Quality Unit Agricultural Research Service, United States Department of Agriculture 950 College Station Road, Russell Research Center, Athens, Georgia 30604

Primary Audience: Broiler Breeder Managers, Researchers, Nutritionists, Poultry Growers

SUMMARY

A study was conducted to examine the effect of yeast culture residue (YCR) on the suppression of aflatoxicosis in broiler breeder hens. One hundred twenty, 35-wk-old, Cobb broiler breeder hens of the same cross were fed diets supplemented with aflatoxin (AF) (0 or 3 mg/kg) and YCR (0 or 2 lb/ton) singly and combined in a 2 × 2 factorial designed experiment. The birds were randomly assigned to pens with 3 replicates of 10 females and 1 male per treatment. Eggs laid by the hens were collected daily, stored at room temperature, and incubated every 7 d for 3 wk. Response variables analyzed were mean percentage of fertility, hatchability, hen-day egg production, egg weight, chick weight at hatch, and embryonic mortality over the 3-wk treatment period. At the end of 3-wk treatment, blood was collected from the hens and analyzed for total protein, globulin, and albumin. Aflatoxin did not negatively affect fertility. However, hen-day egg production (57.6%), percentage of hatchability (67.6%), embryonic mortality (24%), serum total protein, globulin, and albumin were significantly (P < 0.05) affected by AF. Hatch of fertile eggs from the AF-fed hens was significantly lower than the control (67.6 vs. 78.5%). The inclusion of YCR in the AF-treated diet raised the level of hatchability (74.9 vs. 67.6%), egg production (65.83 vs. 57.26%), and lowered embryonic mortality (16.8 vs. 24%). Serum globulin and albumin were lowered in the AF-fed hens but was partially restored with the addition of YCR. The data demonstrated that YCR may enhance the performance of broiler breeder hens that are provided feed contaminated with AF.

Key words: broiler breeder, aflatoxin, live yeast culture

DESCRIPTION OF PROBLEM

Low hatchability is currently a concern in the commercial broiler breeder industry. Studies conducted in the United Kingdom have shown that on average only 83% of broiler breeder eggs produce chicks (as reviewed by Heier and Jarp [1]). A study conducted at the test unit of the

To whom correspondence should be addressed: victor_stanley@pvamu.edu.
Norwegian Poultry Association in 1999 showed an average fertility and hatchability of 86.6 and 72.7%, respectively, in Ross 208 breeders [2]. Developing embryos can be very sensitive to many environmental stresses. These stresses include extremes in temperature control [3] invasion of microorganisms (bacteria and fungi) [4], eggshell quality [5], and water loss from the eggs resulting in dehydration and embryonic death [6]. Other stresses include incorrect hatching egg storage, exposure to chemicals, poor hygiene, damaged eggshells [7], and excessive handling [8].

One critical prehatch stress that can impact hatchability is the consumption of aflatoxin (AF)-contaminated feed [9]. Natural and synthetic compounds have been used as AF binders with varying degrees of success [10]. Binders that have been used as feed additives include hydrated sodium calcium alumina-silicate, zeolites, activated charcoal, and clays, such as bentonite [11]. However, when used at high inclusion rates (1 to 2% of the ration), some binders could dilute nutrient density, whereas other binders do not degrade after they are excreted, therefore, affecting manure lagoons. Clay binders also carry the risk of being contaminated with heavy metals.

Stanley et al. [12] observed that adding live yeast culture (Saccharomyces cerevisiae) to AF-contaminated feed protected broilers from the harmful effects of aflatoxicosis. Later, Stanley et al. [13] investigated the effect of yeast cell wall residue (YCR) on the performance of broiler chicks and found it neutralized the harmful effects that AF produced in broilers. The YCR is the cell wall preparation from yeast (Saccharomyces cerevisiae). It is composed of glucan which consists of several glucose molecules [14]. Glucan is a natural complex carbohydrate that enhances immune response by activating phagocytic cells, which trap and engulf foreign cells [15]. It also has other positive effects on the functioning of the gastrointestinal tract [14]. Glucans are found in the inner cell wall of yeast cells. Another component of YCR is mannan found in the outer cell wall. Mannanoligosaccharide was identified as the active component of the YCR. Because YCR residue was effective in nullifying the severity of aflatoxicosis in broiler chicks to 42 d of age, it was speculated that it could also bind AF in broiler breeder hen diets and protect the hens from the harmful effects of aflatoxicosis. Therefore, the objective of this study was to examine the effects of YCR on the reproductive performance of broiler breeder hens fed a diet containing added AF.

MATERIALS AND METHODS

Birds

One hundred twenty Cobb broiler breeder hens and 12 males, all 35 wk of age, were used to conduct the study. On arrival, the birds were randomly separated into 4 treatment groups, with 3 replications of 10 birds per treatment. Each treatment group consisted of 30 hens and 3 males.

Housing

Birds were placed in an open-sided breeder hen house with a stocking density of 0.81 m²/bird. The house was naturally ventilated through adjustable windows. Before the birds were placed in the pens, the house was cleaned, washed, and disinfected. The birds were placed on concrete floors covered with fresh pine wood shavings. Water was provided for the birds using automatic plastic hanging drinkers with one waterer per pen, and feed was provided using tube-type feeders, one per pen. A 2-tier, 10-hole nest box was provided in each pen. To minimize male effects, the males were rotated weekly among treatment groups.

Preparation and Administration of AF

Biochemical characterization of Aspergillus spp. demonstrated that Aspergillus parasiticus NRRL 2999 was positive for the production of AF [16]. The AF was produced through the fermentation of rice by A. parasiticus NRRL 2999, as described by Kubena et al. [11] and modified by West et al. [17]. The fermented rice was autoclaved and ground to powder, and the AF content was measured by spectrophotometric analysis [18] as modified by Wiseman et al. [19]. The AF within the rice powder consisted of 79% AFB₁, 16% AFG₁, 4% AFB₂, and 1% AFG₂ based on total concentration of AF in the rice powder. The rice powder was incorporated into the basal diet to provide the desired level of 3.0 mg of AF/kg of diet.
Experimental Diets and Treatments

The birds (males and females) were fed a standard broiler breeder ration of corn-soybean base formulated to meet or exceed National Research Council [20] specifications. The CP level of the breeder diet was 16% with 2,900 kcal of ME/kg of feed. All diets were isocaloric and isonitrogenous. Diet 1 was the control diet and contained no AF or YCR [21]. Diet 2 contained AF only (3 mg/kg for feed). Diet 3 contained YCR only (2 lb/ton). Diet 4 contained both AF (3 mg/kg) and YCR (2 lb/ton). Hens were provided the diets ad libitum for 3 wk.

Data Collection

Egg production data were collected daily and recorded as nest, floor laid, cracked, broken, and soft-shelled eggs. Only nest eggs and clean floor eggs were selected for incubation. Dirty, misshaped, broken, cracked, excessively small, and double-yolked eggs were not incubated. All eggs laid were used to evaluate daily hen-day production and mean egg weight.

Eggs were stored at room temperature (72°F) for up to a week and then placed in a Jamesway model 252A incubator [22]. The dry- and wet-bulb temperatures for the incubators (setter and hatcher) were set at 37.6°C and 60% RH. Eggs were separated into compartments in the setter and hatcher so that the exact numbers of hatched chicks could be identified for each replication and treatment. Over the 3-wk period of the study, approximately 42 to 56 hatchable eggs per pen per week were incubated.

On d 18, the eggs were candled to determine macroscopic fertility and early embryonic mortality before being transferred back to an incubator (hatcher) [22] for hatching and were maintained at dry- and wet-bulb temperatures similar to those prior to transfer. Infertile eggs; early, late, and pipped embryonic mortalities; and hatching chick deaths were recorded as part of a hatchery residue analysis. All hatchability and mortality data were expressed as percentages of fertile eggs set. On d 21, chicks were removed from the hatcher, and data on late embryonic mortality and chick weight were collected. Additionally, at the end of the study (3 wk), blood was taken from each hen for serum chemical analysis to determine effects of consumption of diets on blood chemistry of the layer hens.

Statistical Analysis

Data were subjected to a conservative 2 × 2 factorial arrangement of treatments (AF vs. no AF and YCR vs. no YCR) with replications serving as the experimental units. Data were evaluated on egg production, egg weight, fertility, hatchability, early and late embryonic death, and chick weight at hatch. All of the data were analyzed using SAS software [23]. All statements of significance were based on P < 0.05. When data required normalizing, they were converted to the probit scale.

RESULTS AND DISCUSSION

Hen-day egg production (HDP) was significantly reduced when AF was present in the broiler breeder ration (Table 1). The depression in HDP was noted in the AF-treated group and the AF plus YCR-treated group. Figure 1 shows an immediate reduction in the rate of egg production when AF was present in the diet and continued to decrease with succeeding weeks, and the rate of egg production in the controls did not change over time. The data show no significant differences in the settable eggs produced among the treatments (Table 1). However, data reported by Qureshi et al. [9] suggest that AF in the diet could affect the eggshell quality, therefore reducing the number of settable eggs. Extension of the study might support the data presented by Qureshi et al. [9]. The addition of YCR at 2 lb/ton of feed protected the birds from some of the detrimental affects of AF on percentage of egg production (65.83% HDP vs. 57.26% HDP). Hen-day egg production was considerably higher when the breeders were fed diets with no added AF (71.86 and 74.66%). However, egg production by hens provided a combination of YCR and AF was significantly higher than production of AF-fed hens. Wolzak et al. [24] reported that hens fed a diet containing 3,310 µg of AFB1 and 1,680 µg of AFB2/kg of feed for 28 d showed a significant decrease in egg production and egg weight. The reduction in egg production occurred rapidly, reaching maximum levels after 7 d. In the current study, egg production went from 71.86% (controls) to 57.26% (AF-supplemented feed) in 3 wk, but egg production was significantly increased by the addition of YCR at 2 lb/ton of AF-infected feed. The
TABLE 1. Effects of yeast culture residue (YCR) in feed on fertility, hatchability, and chick weight of broiler breeder hens fed diets containing aflatoxin (AF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hen-day production</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AF (mg/kg)</td>
<td>YCR (lb/ton)</td>
<td>Total eggs</td>
<td>Egg production</td>
<td>Settable eggs (%)</td>
<td>Fertility (%)</td>
<td>Hatchability (%)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>150.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>120.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>97.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>156.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>74.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>138.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;B&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Means within columns with no common letter differ significantly (<i>P</i> < 0.05).

<sup>A</sup>Settable eggs included broken, cracked, and soft-shelled eggs.

<sup>B</sup>Pooled SEM.

YCR apparently reduced the deleterious effects of the toxin on egg production. Kubena et al. [11] stated that low BW in broiler chicks receiving AF was associated with low feed consumption. Feed consumption was not measured in the current study. The YCR also has been shown to exhibit physiological effects on microorganisms, such as Salmonella [25] and coliforms in broilers reared on recycled litter [26].

The reproduction data are presented in Table 1. Fertility levels, which were determined by breakout of candled eggs incubated for 18 d, were statistically insignificant for all treatment groups (<i>P</i> > 0.05). On the other hand, hatchability, which represents hatch of total eggs, showed considerable variation. Aflatoxin added to the breeder diet had a detrimental effect on hatchability. Aflatoxin-treated breeders produced a 67.6% hatch compared with the controls with a 78.5% hatch, a difference of 10.9%. The inclusion of YCR in the diet containing AF restored hatchability by 7.3% (67.6 vs. 74.9%). Addition of YCR to a control diet with no AF added to the diet produced no significant increases in hatchability as compared with controls (80.6 vs. 78.5%). Chick weight taken immediately after hatching tended to reflect egg size and was difficult to interpret.
TABLE 2. Effects of yeast culture residue (YCR) in feed on embryonic mortality of broiler breeder hens fed diets containing aflatoxin (AF)

<table>
<thead>
<tr>
<th>Treatment AF (mg/kg)</th>
<th>YCR (lb/ton)</th>
<th>Early death (%)</th>
<th>Late death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>7.1b</td>
<td>8.3b</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>9.0a</td>
<td>15.0a</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>5.7c</td>
<td>7.5b</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>7.7b</td>
<td>9.1b</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>2.95</td>
<td>2.79</td>
</tr>
</tbody>
</table>

Note: Means within columns with no common letter differ significantly ($P < 0.05$).

Data presented in Table 2 show the effects of dietary treatments on early (1 to 18 d) and late (18 to 21 d) embryonic mortality. Feeding AF alone resulted in a significant increase in early and late embryonic deaths. Feeding YCR alone decreased early and late embryonic death equal to levels from eggs laid by hens provided the control diet. Addition of YCR to the diet containing AF significantly lowered early and late embryonic deaths and, therefore, significantly elevated the hatch from hens not provided AF.

Table 3 shows the effects of diets on serum concentrations of total protein, globulin, albumin, calcium, and phosphorus in the blood of the breeder hens. Compared with the control, feeding AF to breeder hens significantly reduced the serum level of total protein, globulin, albumin, calcium, and phosphorus. The addition of YCR to the diet containing AF elevated the serum concentration of total protein, globulin, albumin, and calcium levels that were not significantly different from the control group. Blood serum levels of total protein, globulin, and albumin of AF-fed breeder hens were much reduced compared with the other treatment groups. This result could have been a factor affecting not only rate of egg production but also embryonic mortality. Blood serum albumin appeared to be more reduced than serum globulin as a result of AF in the feed. When YCR was fed along with AF, serum globulin almost returned to control levels. Serum albumin increased but not at the same rate as serum globulin.

Wolzak et al. [24] observed that the transfer of AF toxicity to the eggs occurs rapidly, reaching maximum levels after 4 to 5 d and remains relatively constant throughout a 28-d feeding period. Qureshi et al. [9] reported that developing embryos are very sensitive to AF. In the current study, the ability of YCR to suppress toxicity of AF was demonstrated in improved hatchability when it was added to AF-treated feed. Several studies have shown AF to be immunotoxic in avian species [27]. Studies [28, 29] have shown that AF exposure can affect the development of the immune system during embryonic development. Huff et al. [4] and Kubena et al. [30] also observed liver pathology, immunosuppression, and changes in relative organ weights. When hens consume AF, it is absorbed by the intestine and distributed by the bloodstream throughout the body. Dvorska et al. [31] suggested that YCR prevents changes in fatty acid and antioxidant composition in the egg yolk. Early and late embryonic mortality data showed the severity of AF on embryonic mortality. The inclusion of AF at 3 mg/kg in the diet of our study increased early and late...
embryonic death. The high rate of embryonic death at both stages of incubation, producing low hatchability of eggs from the AF-fed hens, could have been due to low calcium availability. Calcium is vital in the production of strong eggshell. Although eggshell quality was not determined, reduced blood serum calcium and phosphorus could have affected eggshell thickness in the AF-fed breeders. Poor eggshell could interfere with the oxygen-carbon dioxide ratio. Because developing embryos are very sensitive to reduced oxygen supply [7], these changes caused by AF consumption might play a role in increasing the rate of embryonic death.

Although other binders to suppress AF have been suggested, there have been several reported disadvantages. In addition to a low inclusion rate, an ideal AF binder should also be able to bind a wide range of mycotoxins, have a rapid and uniform dispersion, and be heat stable. A good absorbent for AF should have no effect on the absorption of vitamins and other micronutrients by the digestive system and should be biodegradable. The YCR has many desirable attributes as an effective AF binder, and results from the present study indicate that it is effective in increasing hatchability and reducing mortality in eggs from breeders that consume feed contaminated with AF.

CONCLUSIONS AND APPLICATIONS

1. Aflatoxin in a broiler breeder diet at an added level of 3 mg/kg of feed decreased HDP, lowered hatchability, and decreased serum protein, globulin, albumen, calcium, and phosphorus over a 3-wk period, when compared with comparable controls.

2. The addition of YCR to the breeder diet at 2 lb/ton of breeder feed containing 3 mg/kg of added AF significantly improved rate of egg production, hatchability, and certain blood serum components.

3. Yeast culture residue has the potential of binding AF in the short term and minimizing its toxic effects on egg production and hatchability. Its long-term therapeutic effects on the performance of AF-infected birds must be determined.

REFERENCES AND NOTES


reference to production of aflatoxins and cyclophayonic acid. Myco-
pathologia 87:13–15.

yield of aflatoxin by incremental increases in temperature. Appl.


Note on research of pigments from chloroform extracts of aflatoxin
50:982–983.


21. Feed additives used in the experiment were mannanoligosac-

22. Jamesway model 252A incubator, Butler Manufacturing Co.,
Fort Atkinson, WI.


24. Wolzak, A., A. M. Pearson, T. H. Coleman, T. J. Pestka,
and J. I. Gray. 1985. In Aflatoxin Disposition and Clearance in the

mers with significant impact on the gastrointestinal microflora and
the immune system. Pages 167–174 in Annu. Symp. T. P. Lyons and

E. Sefton. 2004. An alternative to antibiotic-based drugs in feed for
enhancing performance of broilers grown on Eimeria spp.-infected

27. Sakhatsky, I. M. 1999. Effect of Fusarium graminearum
Ukraine 9:34–35.

of sister chromatid exchanges by indirect-acting mutagen-carcino-
gens at early and late stages of embryonic development. Environ.

B₁ induced sister chromatid exchanges and cytotoxicity in differenti-

of a hydrated sodium calcium aluminosilicate to reduce the toxicity

2001. Effect of the mycotoxin aurofusarin on the antioxidant compo-