USE OF MOLD INHIBITOR FOR FEED STORAGE AND IMPROVED CHICK PERFORMANCE[1]

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Summary

Two experiments were conducted to evaluate the effect of mold inhibitor in the ration which had two different protein levels (18% and 12%) and two different particle sizes (80 or 40% of the particles in the ration less than 1.19 mm). The experimental diets with ave. 12.7% moisture which were treated at the level of 0.1% mold inhibitor were stored under 85% humidity and at 29 ± 1℃ for 10 to 40 days. In experiment 1, after 40 days of storage the CO_2 production in the feed treated with mold inhibitor was higher ($p < 0.01$) than when 40% of the ration's particle size was 1.19 mm. Aflatoxin production in the experimental diet with mold inhibitor was affected ($p < 0.05$) by the levels of protein and the different particle size ranges after 40 days storage. The interaction of protein levels and particle size ranges on the aflatoxin and CO_2 production was significant ($p < 0.05$) at 40 days storage. In experiment 2, there was a decrease in total body weight gain and total feed intake observed in chicks fed the untreated diet of 18% protein with 40% of the particles in the ration less than 1.19 mm stored for 40 days. Feed conversion was depressed ($p < 0.05$) in the chicks fed the untreated diets of both particle sizes. Particle size X types of feed interaction in feed conversion was significant ($p < 0.05$).

(Key Words: Mold Inhibitor, Protein Level, Particle Size, Aflatoxin, Growth Performance, Organ Weight)

Introduction

Feeds stored under the korean climatic conditions, especially during the rainy season of summer, often become heated and spoil as the result of fungal activity. The typical methods of inhibition of fungal growth have been accomplished by regulating moisture, temperature, and the atmosphere of stored grains and grain products. The method of inhibition most appropriate for feed industries today is the use of chemical preservatives such as propionic, acetic, benzoic, sorbic, and formic acids (Goering and Gordon, 1973; Britt et al., 1975). It, however, appears to be a not uncommon complaint that mold inhibitors do not work consistently and uniformly (Hamilton, 1984).

The effects of various mycotoxins and moldy grain diets on chicks were studied extensively during the last two decades (Frits et al., 1973; Carlton and Krogh, 1979; Pier, 1981; Bartov et al., 1982; Gibson et al., 1989). Bartov et al. (1982) reported that fat content in moistened ground grains decreased markedly during storage, but fatty acid ratios, vitamin E, carotene, xanthophyll, and protein levels were not markedly affected. Although mold inhibitor usage has begun recently in stored feeds, the effect of fungi on the nutritional value of feedstuffs when these inhibitors are used has not received much attention.

Two series of studies were conducted. The first study was undertaken to document the influence of particle size of substrate and the different levels of protein in the commercial mixed diets on the effectiveness of mold inhibitors under conditions similar to the korean rainy season. The second project was conducted to study whether chick performance was adversely affected

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by feedstuffs stored for 40 days mixed with mold inhibitors compared to a similar untreated diet.

Materials and Methods

Diet preparation

The experiments were conducted on the effect of particle size of two diets with different levels of protein (18% in the chick starter and 12% for the beef ration which contained 12.6 and 12.7% moisture content respectively, table 1) on the inhibitor and the interactions between particle size and protein levels on the inhibitor.

TABLE 1. COMPOSITION OF TWO DIETS

Item	Composition (%)	
	Chick starter	Beef ration
Corn, yellow	36.0	35.0
Sorghum	9.0	
Wheat	16.8	27.0
Soybean meal (44%)	16.8	
Rapeseed meal	2.5	0.63
Wheat bran	6.37	19.0
Defatted rice bran	4.0	10.0
Fish meal (62%)	4.0	
Urea		0.42
Molasses		3.5
Limestone	1.2	4.0
Bone meal	2.0	
Salt	0.2	
Premix	1.13[1]	0.4
Calculated analysis:		
Moisture	12.6	12.7
Crude protein	18.0	12.0

[1] Supplied the following per kg of starter: Vitamin A, 8,800 IU; Vitamin D_3, 2,200 ICU; Vitamin E, 5.5 IU; Menadion Sodium Bisulfite, 3.33 mg; Thiamine, 1.1 mg; Ribollavin, 6.6 mg; Pantothenic acid, 11 mg; Niacin, 3.3 mg; Choline, 385 mg; Vitamin B_{12}, 0.011 mg; Folic acid, 0.77 mg; Pyridoxine, 1.1 mg; Biotin, 0.22 mg; Manganese, 68 mg; Zinc, 55 mg; Iron, 26 mg; Copper, 4.4 mg; Iodine, 1 mg.

After formulating each ration (chick starter and beef ration) main ingredients of each ration were ground in a Wiley Mill and seperated into two particle size ranges (larger than 1.19 mm and less than 1.19 mm) using a U.S. standard mesh screen. The feed ingredients were then mixed to form chick starter and beef rations. In this mixing, the particle sizes of the diets were manufactured as follows: (1) 80% of the particles in the experimental ration less than 1.19 mm, (2) 40% of the particles in the experimental ration less than 1.19 mm.

The experimental diets treated and untreated with mold inhibitors were divided into four plastic cans of 3 kg for each treatment. The mouth of each plastic can was the same size as its base. The plastic can was covered but not sealed hermitically, thereby permitting an adequate exchage of air and humidity for mold growth. The plastic cans were placed in the incubator which was converted from an egg incubator with an evaporate, forced air humidifier set at 85% humidity and a controlled-temperature of 29 ± 1℃. Relative humidity was measured with a wet bulb thermometer. Rations were incubated for 10, 20 and 40 days. After incubation for 10, 20 or 40 days, 250 g of sample were taken from each plastic can and transported to the laboratory in plastic bags and frozen at −25℃ until analyzed.

Inhibitor

The mold inhibitor studied was MYCO CURB (Kemin Industries Inc., Des Moines, Iowa, U. S. A.). MYCO CURB, which is a commercial inhibitor widely used in the feed industry in Korea, contains a few organic acids distributed in a finely divided calcium silicate carrier, and it is markcted as a fine powder.

MYCO CURB was mixed in the treated diets at the level of 0.1% (w/w) which was the recommended level by Kemin Industries Inc.

Chemical method for feed sample analyses

Moisture content of the feed was determined gravimetrically after heating 10 ± 0.01 g in an oven at 100 ± 2℃ for 18 to 20 hrs. After removing plastic cans from the incubator, plastic tubing was inserted to the center of each plastic storage can and air samples were withdrawn by hand-pump and flushed through glass vials which were then sealed and taken to the laboratory. Gas samples were removed from the vials by syringe through a septum (Paster, 1979) and were then analyzed by gas chromatography using the

method described by Navarro and Donahaye (1972).

The assay for aflatoxin B_1, B_2, G_1 and G_2 was carried out using the AOAC method 975.36 (AOAC, 1990) and the assay for zearalenone was done with the modifications of Eppley (1968) and Howell and Taylor (1981), as follows: A ground sample of 50 g was weighed out and extracted with 150 ml of methanol-water (90:60) for 5 min. Filtration was carried out using Whatman #4 filter paper. The filtrate was added to 100 ml of hexane two times. The mixture of the filtrated and hexane was swirled and the hexane layer was discarded. Iron hydroxide gel was added to the hexane layer for further clean up and it was allowed to stand for about 5 to 10 min. It was filtered again through Whatman #4 filter paper. 1.5 ml of 3 M HCl was poured into the filtrate in a 250 ml seperatory funnel and the seperatory funnel was rotated for 2 min. The mixture was extracted into a 100 ml beaker and evaporated to near dryness. The wall of the beaker was rinsed with a few drops of dichlormethane-methanol (3:1) and transferred to a test tube. The residue in the test tube was evaporated using a N-evaporator to less than 0.5 ml. The sample in the test tube was loaded on the precoated silica gel 60 thin layer chromatography (TLC) plates by using a micro-syringe. Plates were developed in benzene: methanol (97:3, v/v) for zearalenone. The plates were viewed, after drying, under ultraviolet light for flourescent spots and Rf typical to zearalenone.

Chick performance

Day-old Hubbard male chicks were raised in electrically heated battery brooders. After a preparatory period of 7 days, during which time the chicks were fed a commercial starter diet, the chicks were wing-banded and divided into 6 treatment with 4 replicates per treatment and 10 birds per replicate. After sampling from each plastic can for nutrient analysis only the chick starters with 18% protein from the incubator were used in the feeding experiment for the investigation of chick performance. The composition of the basal diets which were used in the feeding experiment is presented in table 1.

The experiment diets were fed *ad libitum* in mash form for three weeks, up to 28 days of age. Body weights were recorded weekly on an individual basis. Feed consumption data was obtained at weekly intervals on a group basis.

At 28 days of age, all birds were killed by cervical dislocation, and the weights of the liver, pancreas and spleen were recorded. The chicks selected for these determinations represented the average body weight of the corresponding treatments.

Statistical analysis

In experiment 1, a 2 × 2 factorial experimental design (two levels of protein and two different particle sizes) was composed of 4 treatments and 4 replicates per treatment. In experiment 2, a 2 × 3 factorial experiment (two levels of particle sizes and three types of feed) was carried out. Results from experiment 1 and 2 were subjected to analysis of variance (Steel and Torrie, 1980) and to Duncan's multiple range test (Duncan, 1955).

Results and Discussion

Experiment 1

Accumulation of CO_2 in the plastic cans filled with untreated feed and in plastic cans containing feed treated with mold inhibitor after 10, 20 and 40 days of storage in shown in table 2.

Content of CO_2 in feed treated with mold inhibitor was uniformly low (below 0.5%) up to 40 days of storage, whereas in the untreated feed CO_2 concentration reached a peak of 10.9% after 40 days. Production of CO_2 has been used to evaluate mold inhibitors in silage (Daniel et al., 1970), in storage tanks of poultry feed (Paster, 1979), and in the evaluation of some organic acids as mold inhibitors (Dixon and Hamilton, 1981[a]).

Table 2 did not show any significant differences ($p > 0.05$) in the production of CO_2 between 18% and 12% protein levels in the feeds treated with mold inhibitor. This result was similar to the results of the study of Paster et al. (1987) who researched the antifungal activity of calcium propionate (0.3%), Agrosil (0.2%), and liquid or powdered Adofeed (0.2%) in poultry feed. They found that carbon dioxide started to accumulate in control bins after 15 days storage and in bins treated with calcium propionate or powdered Adofeed after 40 days of storage. There were no differences ($p > 0.05$) in their research on the

TABLE 2. CARBON DIOXIDE CONCENTRATIONS AT 10, 20 AND 40 DAYS STORAGE

Protein	1.19 mm particle	Moisture content	CO_2 concentration (%)					
			10 days		20 days		40 days	
(%)	size (%)	(%)	UT[1]	T	UT	T	UT	T*
18	80	12.6	5.50	0.29[A]	6.10	0.27[A]	10.40	0.29[A]
	40	12.6	5.26	0.39[B]	6.00	0.42[B]	10.50	0.41[B]
12	80	12.7	5.80	0.25[A]	6.50	0.25[A]	10.90	0.27[A]
	40	12.7	5.30	0.41[B]	6.00	0.41[B]	10.50	0.47[B]
Mean protein (%)	18		5.38	0.34	6.05	0.35	10.45	0.35
	12		5.55	0.33	6.25	0.33	10.70	0.37
1.19 mm particle size (%)	80		5.65	0.27[C]	6.30	0.26[C]	10.65	0.28[C]
	40		5.28	0.40[D]	6.00	0.42[D]	10.65	0.44[D]

[1]UT: Untreated diet. T: Treated diet.
[A,B] Values with different superscripts within the 4 treatments are significantly different ($p < 0.05$).
[C,D] Values with different superscripts within the different particle sizes are significantly different ($p < 0.01$).
* Protein × particle size interaction was significant ($p < 0.05$).

production of CO_2 between 18% and 12% protein levels in the feeds treated with mold inhibitor.

Table 2 indicated that the particle sizes of the substrate for fungal activity had an influence on the inhibitory properties of the mold inhibitor ($p < 0.01$). The effect of particle size was such that the smaller the particle size of the ration the greater the activity displayed by the mold inhibitor. Dixon and Hamilton (1981[b]) showed that the inhibition of propionic acid was size dependent at the three H_2O levels treated (20, 25 and 35%) and at the higher concentration of propionic acid (1.0 and 2.0 mg/g of meal). Their findings and the current research results may be

TABLE 3. MYCOTOXIN (AFLATOXIN B_1) PRODUCTION AT 10, 20 AND 40 DAYS STORAGE

Protein	1.19 mm particle	Moisture content	Mycotoxin production (mcg/kg)						
			Fresh	10 days		20 days		40 days	
(%)	size (%)	(%)	feed	UT[1]	T	UT	T	UT	T*
18	80	12.6	0.0	2.2	0.0	2.7	1.3	6.8	4.5[A]
	40	12.6	0.0	2.0	0.0	3.0	1.1	6.8	4.9[A]
12	80	12.7	0.0	2.1	0.0	2.8	1.1	6.4	3.6[B]
	40	12.7	0.0	2.4	0.0	2.9	1.3	6.5	4.5[A]
Mean protein (%)	18		0.0	2.1	0.0	2.8	1.2	6.8	4.7[C]
	12		0.0	2.3	0.0	2.9	1.2	6.5	4.1[D]
1.19 mm particle size (%)	80		0.0	2.2	0.0	2.8	1.2	6.6	4.0[E]
	40		0.0	2.2	0.0	3.0	1.2	6.4	4.7[E]

[1]UT: Untreated diet. T: Treated diet.
[A,B] Values with different superscripts within the 4 treatments are significantly different ($p < 0.05$).
[C,D] Values with different superscripts within the different particle sizes are significantly different ($p < 0.05$).
[E,F] Values with different superscripts within the different particle sizes are significantly different ($p < 0.05$).
* Protein × particle size interaction was significant ($p < 0.05$).

explained by the fact that dispersion of inhibitor in the substate and the ability of an inhibitor to penetrate the substrate are important attributes of mold inhibitors. In the present study protein levels X particle size was significant ($p < 0.05$) in the feed stored for 40 days (table 2).

None of the rations in the present study contained detectable amounts of aflatoxin B_2, G_1, G_2 and zearalenone (table 3).

A trace of aflatoxin B_1, however, was formed after 10 days of storage in the untreated ration under the condition of 29 ± 1℃ and 85% relative humidity. After 40 days of storage the concentration of aflatoxin sharply increased. In samples treated with mold inhibitor, aflatoxin production started from 20 days of storage. After 40 days storage the concentration of aflatoxin found in the treated feed was over four times that found in the feed stored for 20 days. table 3 shows that the concentration of aflatoxin in the treated feed which contained 12% protein and had 80% of the particles below 1.19 mm was the lowest level, 3.6 mcg/kg, among treatments ($p < 0.05$). The present results show that feed which contains high protein-containing substances would be expected to neutralize acids such as mold inhibitors (Tabib et al., 1981).

The different particle sizes and protein levels in feed affected ($p < 0.05$) the formation of aflatoxin after 40 days of storage (table 3). Schroeder (1969) stated that the three most important factors influencing aflatoxin formation in stored field crops were moisture, relative humidity, and temperature. And it is generally accepted that feed stored below 14% moisture will not permit the growth of A. flavus and A. papasiticus, but the present study shows that this assumption is clearly untrue. The production of aflatoxin in the complete rations of the current research appeared at 12.7% moisture content and 85% relative humidity, agreeing with the reports of Jones et al. (1982) and under the temperature of 29 ± 1℃, which was higher than those reported for stored grain (Schroeder and Hein, 1967 ; Jones et al., 1982). In the current research aflatoxin production was affected ($p < 0.05$) by the protein levels in the rations while CO_2 production was not affected by the protein levels in the ration (table 2, 3).

Experiment 2

The results of total body weight gain, feed intake and feed efficiency during the experimental period are summarized in table 4.

A significant decrease ($p < 0.05$) in total body weight gain and total feed intake was observed in chicks fed the untreated diet with 40% of the ration less than 1.19 mm. Feed conversion was significantly ($p < 0.05$) depressed in the chicks fed the untreated diets of both particle sizes. Particle sixe x types of feed interaction in feed conversion was significant ($p < 0.05$). It has been widely reported that some nutritional deficiencies, especially most essential animal acid deficiencies,

TABLE 4. EFFECT OF POULTRY DIETS STORED FOR 40 DAYS TREATED OR UNTREATED WITH MOLD INHIBITOR ON THE PERFORMANCE OF 28-DAY-OLD MALE CHICKS

Treatment	Body weight gain (g/chick)	Feed intake (g/chick)	Feed conversion* (feed/gain)
HF[1]	666.7^{B}	803.3^{B}	2.33^{B}
HT	655.8^{B}	810.5^{B}	2.34^{B}
HNT	644.6^{AB}	785.7^{AB}	2.42^{A}
LF	656.6^{B}	813.4^{B}	2.32^{B}
LT	656.8^{B}	793.4^{B}	2.36^{B}
LNT	633.3^{A}	778.8^{A}	2.39^{A}

[1]H: 80% of the experimental diet's particle size less than 1.19 mm.
L: 40% of the experimental diet's particle size less than 1.19 mm.
F: Fresh diet T: Treated diet NT: Untreated diet
^{AB} Values with different superscripts within the 6 treatments are significantly different ($p < 0.05$).
* Particle size × feed types interaction was significant ($p < 0.05$).

in the moldy grain and mycotoxin from the moldy grain can result in the retardation of growth and the lower utilization of feed (Richardson *et al.*, 1962; Fritz et al., 1973; Carlson and Krogh, 1979; Pier, 1981; Bartov et al., 1982; Gibson et al., 1989).

Relative sizes of the liver, pancreas and spleen were not significantly different ($p > 0.05$) among the treatments (table 5).

Aflatoxin increased liver and pancreas weights (Smith and Hamilton, 1970; Doerr et al., 1983; Kimbol and Doerr, 1987; Merkley et al., 1987). The amount of aflatoxin in the untreated diet after 40 days storage was 6-7 mcg/kg as was indicated in the first part of this research. The aflatoxin levels in the present research diet compared with those of the above researchers were not enough to show an increase in organ weight.

The results of these studies suggested that the particle sizes of feed and the levels of protein in the rations are able to affect the utilization of mold inhibitors which are mixed in the ration.

TABLE 5. WEIGHT OF LIVERS, KIDNEYS AND SPLEENS OF 28-DAY-OLD MALE CHICKS FED A 18% PROTEIN DIET FERMENTED FOR 40 DAYS

Treatment	Liver weight (g/kg B. Wt.)	Pancreas weight (g/kg B. Wt.)	Spleen weight (g/kg B. Wt.)
HF[1]	25.4	2.2	1.0
HT	24.9	2.0	1.0
HNT	25.1	2.1	1.0
LF	25.0	2.2	1.1
LT	25.4	2.1	1.0
LNT	24.0	2.2	1.1

[1]H: 80% of the experimental diet's particle size less than 1.19 mm.
L: 40% of the experimental diet's particle size less than 1.19 mm.
F: Fresh diet T: Treated diet NT: Untreated diet

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