MILWHITE'S JOURNAL

ISSUES 1-28

(August, 2014 - November, 2016)

MILWHITE'S JOURNALS

Milwhite's Journal is a monthly communication started in August, 2014 in order to provide the clients of Milwhite, Inc. and others with information about numerous topics of interest within the poultry industry as well as within the overall animal industry. The information is presented in a short easy to read format and covers, not only information about specific Milwhite, Inc. products such as IMPROVED MILBOND-TX®, but also topics often discussed in the areas of animal nutrition, management, production and health. The following are the titles of the 28 journal articles which are found in this booklet. This information and future information to be published in Milwhite's Journal can be found after the first of each month by clicking on "Animal Health Solutions" located on the first page of the Milwhite, Inc. webpage and then clicking on "Latest Journal".

TITLES OF THE FIRST 28 MILWHITE'S JOURNAL ARTICLES

1) Milwhite Launches Monthly Journal
2) IMPROVED MILBOND-TX®: "Safety Studies with Broilers", Body Weight, Feed Consumption, Feed Conversion & Excreta Moisture
3) IMPROVED MILBOND-TX®: "Safety Studies with Broilers" Toe Ash and Blood Serum Components"
4) IMPROVED MILBOND-TX®: "Safety Study With Egg-Type Laying Hens"
5) IMPROVED MILBOND-TX®: "Safety Study With Variable Energy Levels"
6) IMPROVED MILBOND-TX®: "Safety Study With Broiler Breeders"
7) IMPROVED MILBOND-TX®: "Safety Study With Low Phosphorus Levels"
8) IMPROVED MILBOND-TX®: "*In-vivo* Aflatoxin B_1 Binding in Pigs"
9) IMPROVED MILBOND-TX®:"Safety Study Demonstration No Buffering Capacity"
10) IMPROVED MILBOND-TX®: "*In-vitro* Aflatoxin B_1 Binding Capability"
11) IMPROVED MILBOND-TX®: "*In-vivo* Aflatoxin B_1 Binding Capability"
12) IMPROVED MILBOND-TX®: "Reduction of Aflatoxin Transfer From Feed To Milk"
13) IMPROVED MILBOND-TX®: "Aflatoxin And Liver Flukes In Dairy Calves"
14) IMPROVED MILBOND-TX®: CLAY-BASED MYCOTOXIN BINDERS: "Understanding Clays: Their Origin, Formation & Location"
15) IMPROVED MILBOND-TX®: CLAY-BASED MYCOTOXIN BINDERS:"Reasons Why IMTX Is An Efficient AFB_1 Binder"
16) IMPROVED MILBOND-TX®:MYCOTOXIN BINDERS: "All Clay-Based Additives Are Not The Same"
17) IMPROVED MILBOND-TX®: VANADIUM: KEEP IT OUT OF LAYING HEN DIETS
18) IMPROVED MILBOND-TX®: VANADIUM: A FACTOR RESPONSIBLE FOR DEPIGMENTATION OF BROWN SHELLED EGGS
19) IMPROVED MILBOND-TX®: "Overcoming the Detrimental Effects that Vanadium has on Brown Egg Shell Pigmentation"
20) IMPROVED MILBOND-TX®: Why Brown Eggs Shell Pigmentation Decreases with Age of a Layer Flock"
21) IMPROVED MILBOND-TX®: UNDERSTANDING PHOSPHATE SOURCES: "Removing a Major Misconception About Feed –Grade Phosphates"
22) IMPROVED MILBOND-TX®: UNDERSTANDING FEED-GRADE CALCIUM PHOSPHATE SOURCES: "A Brief General Overview About Their Production"
23) IMPROVED MILBOND-TX®: UNDERSTANDING FEED-GRADE CALCIUM PHOSPHATE SOURCES: "Tri-Calcium –Phosphate" (More Than Just A Source Of Phosphorus)
24) IMPROVED MILBOND-TX®: "UNDERSTANDING FEED-GRADE CALCIUM PHOSPHATE SOURCES: High Dietary Levels Of Monobasic Or Dibasic Phosphate Affect Laying Hens Differently"
25) IMPROVED MILBOND-TX®: "Assisting Poultry Nutritionally in the Presence of Molds & Mycotoxins."
26) IMPROVED MILBOND-TX®: "PROMOTES INTESTINAL HEALTH"
27) IMPROVED MILBOND-TX®:"PROMOTES INTESTINAL HEALTH: Understanding the Mechanism of Action."
28) IMPROVED MILBOND-TX®: "Strategies exist for Coping with poor Quality Grain: PART 1"

***WEBSITE: www.milwhite.com**

MILWHITE INC.
JOURNAL

AUGUST/2014

MILWHITE LAUNCHES MONTHLY JOURNAL

Milwhite Inc. has always strived to provide our client's needs in a timely and professional manner with a product that is of the highest quality. These needs are not only provided by our products from a physical / functional perspective but are often in the form of answers to questions about the specific products we produce as well as information about particular issues existing today within the dynamic feed and animal industries. It is because of the desire for information and requests during the past few years by many of our clients worldwide that Milwhite Inc. is launching a monthly communication, ***Milwhite's Journal***. By providing this communication of a one page format, our clients will quickly be brought up-to-date about our products as well as many of the topics often discussed in the areas of animal nutrition, management, production, health, etc.

ABOUT THE AUTHOR

The person responsible for putting together this monthly communication and organization of its content will be Dr. Orlando Osuna, Director of Health Services for Milwhite, Inc. Also, Dr. Richard Miles, Professor Emeritus at the University of Florida will assist in the development of publications. Both are well known nationally and globally and are very capable of compiling short, easy to read, meaningful contributions to ***Milwhite's Journal.***

WHAT TO EXPECT

The first series of monthly communication in ***Milwhite's Journal*** will be dedicated to one of our most successful products, Improved Milbond-TX ® (IMTX). The development of IMTX from the original MTX took two years of intensive *in-vitro* and *in-vivo* testing to prove its efficacy and safety as a mycotoxin binder with a high affinity for aflatoxin and fumonisin. Milwhite, Inc. introduced IMTX to the feed and animal industries in 1992. Since its introduction as a mycotoxin binder, IMTX has been continually tested in animal studies involving broilers, egg-type laying hens, broiler breeders, piglets, dairy cows and salmon. To date, over 30 *in-vivo* studies have been conducted in 10 major universities located mostly in the United States. Data collected in these studies have not only provided required evidence of the efficacy and safety of using IMTX in animal diets, but beneficial expectations anticipated from dietary supplementation of this inert clay-based adsorbent. The university safety studies and the studies proving the efficacy of IMTX as a mycotoxin binder will be the focus of the first issues of ***Milwhite's Journal.*** In subsequent issues, the wide-ranging additional benefits of adding IMTX to diets will be discussed along with many general and specific key topics concerning various areas of animal nutrition, management and production.

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MILWHITE INC.
JOURNAL

SEPTEMBER/2014

IMPROVED MILBOND-TX ®

"SAFETY STUDIES WITH BROILERS"

BODY WEIGHT, FEED CONSUMPTION, FEED CONVERSION & EXCRETA MOISTURE

Improved Milbond-TX ® (IMTX) has been supplemented to poultry diets successfully since 1992 when it was first introduced to the animal feed industry by Milwhite, Inc. The recommended concentration of IMTX in feed is 0.25% (2.5 g/kg diet) and no safety issues, with respect to animal performance, have ever been documented when fed at this concentration. IMTX is an inert montmorillonite clay-based adsorbent originating from natural clay deposits mined directly from the earth. Even though in 1999 Dr. David Ledoux, at the University of Missouri (USA), reported that IMTX was safe when used at higher than recommended dietary concentration in broiler diets, Milwhite Inc. decided in 2006 to initiate studies in which specifically designed experiments were conducted by Dr. Richard Miles at the University of Florida (USA), that would ascertain the safety of IMTX in poultry diets when used at its recommended concentration and up to eight times that concentration. The goal of Milwhite, Inc. in having these studies conducted was to provide clients in the poultry and feed industry with evidence, gained by data collected in well-designed experiments, that if a feed mixing error occurred and IMTX was added to a diet at higher than its recommended concentration it would be completely safe and birds would perform normally. The studies demonstrating the safety of IMTX when fed at high dietary concentrations is the topic of this and other issues of "***Milwhite's Journal***" which will be published in the upcoming months.

THE FACTS

In experiment 1, conducted at the University of Florida (Miles and Henry, 2007), broiler diets were formulated to be isocaloric and isonitrogenous and contained IMTX at concentrations of 0, 0.25, 0.50, 1.50 or 2.0 %. In the second experiment, in order to mimic a feed mixing error, these investigators decided to add IMTX directly to the finished feed (by weight) at concentrations of 0, 1.0 or 2.0 %. In both experiments, in order to eliminate extremes in initial pen body weights, five Ross X Ross male broiler chicks were selected by weight from a larger group and allocated randomly to pens in Petersime battery brooders in an environmentally controlled room with constant lighting. Dietary treatments were fed to 16 replicate pens each containing five chicks. All birds were offered diets and water *ad-libitum* throughout the entire 21-day experimental period. During the last 7 days of each experiment, excreta were collected separately for 48 hours in aluminum pans placed directly under each pen of birds. Following collection, each excreta sample was homogenized by hand for 2 minutes in a 2 L beaker using a spoon. Three sub-samples of approximately 2 grams each were used for moisture determination.

At all dietary concentrations of IMTX body weight of chicks at the end of each 7 days was similar to the control group of birds fed diets containing no supplemental IMTX. Feed consumption and feed conversion followed the same trend in both experiments with no significant differences observed among the dietary treatments. Excreta moisture was not affected in a negative manner (higher moisture content) at any dietary concentration of IMTX. In both experiments, the survivability of chicks was above 98%. Data collected in these two experiments, specifically designed to study the safety of IMTX when supplemented at higher than the recommended concentration, confirmed the fact, that IMTX is indeed safe to add to broiler diets at concentrations up to 2.0%, which is eight times the recommended amount to be used in a diet.

In the previously mentioned safety study which was conducted at the University of Missouri by Dr. Ledoux, these researchers (Ledoux et al., 1999) fed a diet containing 1.0% IMTX to broilers and no detrimental response in bird performance was reported. It was reported in their publication that IMTX was totally effective in removing the toxic effects in broilers from feeding 4 mg/kg of aflatoxin B_1 and no detrimental effects in broiler performance resulted from supplementing IMTX to the diet at a concentration of 1.0% which is four times the recommended amount of 0.25%.

The information presented in this issue of MILWHITE'S Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Miles, R.D. and P.R. Henry. 2007. Safety of Improved Milbond-TX® when fed in broiler diets at greater than recommended levels. Animal Feed Science and Technology. 138:309-317.

Ledoux, D.R., G.E. Rottinghaus, A.J. Bermudez and M. Alonso-Debolt. 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poultry Science. 78:204-210.

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MILWHITE INC.
JOURNAL
OCT/2014

IMPROVED MILBOND-TX®

SAFETY STUDIES WITH BROILERS "TOE ASH AND BLOOD SERUM COMPONENTS"

PREFACE

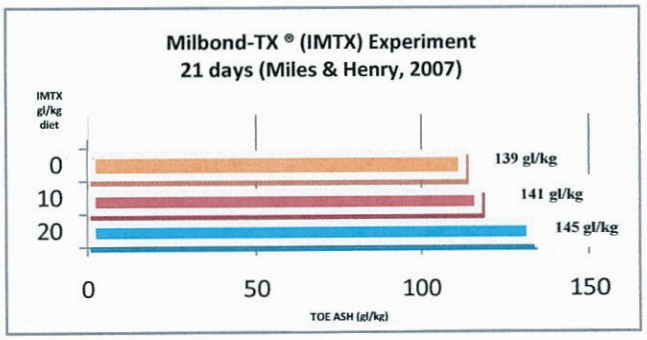
Supplementing Improved Milbond-TX® (IMTX) to broiler diets at higher than the recommended concentration of 0.25% (2.5 g/kg diet) has been reported to be safe and no detrimental effects on broiler performance have occurred even when diets containing up to 2.0% IMTX are fed. This was confirmed in two experiments by researchers at the University of Florida (Miles and Henry, 2007) when they reported that feeding IMTX in the diet of broilers at concentrations up to 2.0% did not have any detrimental effect on broiler performance as measured by body weight, feed consumption, feed conversion and excreta moisture. Also, in these experiments, the authors collected data on percent toe ash and components of serum chemistry in their birds at the end of the 21 day experimental period. In broilers fed diets containing up to 2.0% IMTX the percent toe ash and components of serum chemistry were no different than those for the control broilers fed diets containing no IMTX.

TOE ASH

Miles and Henry (2007) selected toe ash as the well-established method of determining bone mineralization rather than tibia ash because of the simplicity of toe collection and preparation compared with that required for bone ash. Hence, no ether extraction or removal of flesh tissue is required when using toe ash as the measure of bone mineralization. At the end of the 21-day experimental period for each experiment all chicks were killed and the middle toes of each chick were removed at the tarsometatarsal/P3 joint with the skin intact. The tip of the toe containing the nail was removed and discarded, and then the toes were cleaned of any adhering foreign material with a wet paper towel and pooled by pen for toe ash analysis. Each set of toes was dried in an oven for 48 hours at 100°C, then ashed in a muffle furnace at 550°C for 14 hours to determine the toe ash weight (see figure).

BONE MINERALIZATION

Bone mineralization was of special importance in these experiments in which high dietary concentrations of IMTX were fed to broilers since Aluminum (Al) is one factor that has a profound negative effect on bone mineralization and IMTX contains from 145 to 197 g Al_2O_3. (100 to 136 g/kg Al)/kg. It is well established that Al complexes with P in the gastrointestinal tract of animals and reduces the relative bioavailability of P. If the Al in the IMTX complexed with P in the gastrointestinal tract and prevented absorption and utilization of P in bone formation, then a decrease in serum P and toe ash would be expected. This would be especially true with the highest concentration of IMTX in the diet. These negative effects were not observed in these experiments discussed by Miles and Henry (2007).



BLOOD SERUM COMPONENTS

In the in-vivo study reported by Ledoux et al. (1999) serum chemistry profile was of major concern since their broilers were being fed diets containing 4 mg/kg aflatoxin B_1 with and without IMTX at a dietary concentration of 1%. When the serum chemistry values for Ca, P, Na, Cl, total protein, cholesterol, glucose, albumin and globulin, in the chicks fed IMTX with no aflatoxin B_1, were compared to values for chicks fed the control diet also containing no aflatoxin B_1, similar values existed. Also, IMTX was shown in these experiments to be 100% effective in removing the negative effects on broiler performance of feeding diets containing 4 mg/kg aflatoxin B_1. In addition, except for glucose and cholesterol, IMTX restored back to control diet values the serum chemistry profile values of chicks fed the diet containing the combination of aflatoxin B_1/IMTX. Thus, these authors were able to demonstrate that IMTX fed at a dietary concentration of 1.0% was totally safe to broiler performance and also completely removed the negative effects in broilers fed diets containing 4 mg/kg aflatoxin B_1.

In the IMTX safety experiments reported by Miles and Henry (2007)the serum chemistry values for Ca, P, Na, Cl, K, total protein, triglycerides, cholesterol, glucose, albumin and uric acid in control birds fed no IMTX were no different than those from broilers fed diets containing IMTX at a concentration of 2.0%. The dietary concentration of IMTX used by these investigators was twice the concentration used in the study reported by Ledoux et al. (1999) in which they also reported no detrimental effect on broiler performance of feeding IMTX at a dietary concentration of 1.0%.

SUMMARY

The studies reported here which were conducted at the University of Florida (USA) and the University of Missouri (USA)clearly demonstrate that when IMTX is fed to broilers at a dietary concentration that is eight times greater than the recommended concentration of 0.25%, birds performed the same as when fed diets containing no IMTX. This is not surprising since IMTX is inert and not expected to affect animal performance.

The information presented in this issue of MILWHITE'S Journal was complied by Dr. Orlando Osuna, Director of Heath Science at Milwhite, Inc. & Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.
Miles, R.D. & P.R. Henry 2007. Safety of Improved Milbond-TX ® when fed in broiler diets at greater than recommended levels. Animal Feed Science and Technology. 138:309-317.
Ledoux, D.R., G.E. Rottinghaus, A.J. Bermudez and M. Alonso-Debolt. 1999. Efficacy of a hydrated sodium calcium aluminosilcate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poultry Science. 78:204-210.

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MILWHITE INC
NEWSLETTER

NOV/2014

IMPROVED MILBOND-TX ®

"SAFETY STUDY WITH EGG-TYPE LAYING HENS"

PREFACE

In experiments specifically designed to establish the safety of Improved Milbond-TX® (IMTX) in broiler diets containing higher than the recommended dietary concentration of 0.25% (2.5 g/kg diet), researchers (Miles and Henry, 2007a: Ledoux et al., 1999) reported no detrimental effects on broiler performance. Their findings, from feeding up to 2.0% IMTX in broiler diets, were presented in summarized form in two of the past issues of "***Milwhite's Journal***". Following this research on safety of IMTX in broiler diets an experiment was designed to establish the safety of IMTX fed at up to 2.0% in commercial egg-type laying hen diets. The study was conducted at the University of Florida using Hy-Line W-36 hens fed a typical corn-soybean meal based diet. The following is a brief summary of this safety study.

EXPERIMENTAL DESIGN

Researchers conducting this study (Miles and Henry, 2007b) decided not only to establish the safety of IMTX in laying hen diets, but to design the study to determine if feeding high dietary concentrations of IMTX affected hens known to be laying eggs with high shell weight or low shell weight. Prior to feeding high dietary concentrations of IMTX to hens the researches (Miles and Henry, 2007b) separated the hens into two groups according to their shell weight. Three eggs were collected from each of 300 individually caged, thirty-five-week old hens. The eggs were weighed, broken out, washed of excess albumen and dried to determine the average shell weight for each individual hen. All hens were ranked by shell weight based on mean shell weight of the 3 eggs collected. The 75 hens with the highest and 75 hens with the lowest shell weights were allocated randomly to 5 replicate pens of 5 individually caged birds with either high or low shell weight for the experiment. Each pen of 5 hens was assigned randomly to 1 of 3 dietary treatments, which were the corn-soybean meal basal diets supplemented with 0, 1.0 or 2.0% IMTX. Feed and water were offered *ad-libitum* to the hens throughout the 5 28-day experimental periods. Data on bird performance and egg characteristics were collected at pre-determined days during each 28-day experimental period. Also, during the fourth 28-day period, excreta were collected from all replicate pens of five hens for moisture determination.

RESULTS

When the data were collected and analyzed for this study, IMTX fed at concentrations higher than the recommended amount in the diet proved to be safe and did not affect any component of laying hen performance. Hen mortality was less than 3% during the entire experimental period and was not related to any dietary treatment. Feed intake, feed conversion, and hen-day egg production in both groups of hens selected for their eggshell weight was not influenced by the addition of IMTX to the diet. Change in hen body weight during the five-month experimental period did not differ among treatments for hens in the shell weight groups, nor was there an effect due to IMTX. Egg characteristics (egg weight, shell weight and Haugh units (albumen quality) were not affected by IMTX during the entire 20 week experimental period. Values for excreta moisture of hens in the shell weight groups were similar and IMTX did not have any influence on excreta moisture.

CONCLUSIONS

The data collected in this experiment with commercial egg-type laying hens, support the conclusion obtained with broilers, that feeding higher than recommended amounts of IMTX in the diet is safe. No detrimental effects on laying hen performance or egg characteristics resulted from feeding up to 2.0% IMTX in the diet continuously for 5 months to commercial egg-type laying hens grouped according to their ability to lay eggs with high or low egg sheli weight.

The information presented in this issue of Milwhite's Journal was complied by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Miles, R.D. and P.R. Henry. 2007a. Safety of Improved Milbond-TX® when fed in broiler diets at greater than recommend levels. Animal Feed Science and Technology.138:309-317

Miles, R.D. and P.R. Henry. 2007b. Safety of Improved Milbond-TX® when fed to laying hens at higher-than-recommended levels. Journal of Applied Poultry Research. 16:404-411

Ledoux, D.R., G.E. Rottinghaus, A.J. Bermudez and M. Alonso-Debolt. 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poultry Science 78:204-210

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MILWHITE, INC.
JOURNAL

DECEMBER/2014

IMPROVED MILBOND-TX®

"SAFETY STUDY WITH VARIABLE ENERGY LEVELS"

In 1999, Dr. David Ledoux and coworkers at the University of Illinois (USA) conducted a safety study with Improved Milbond-TX (IMTX), in which they supplemented broiler diets at a dietary concentration of 1.0% (10g/kg diet), which was four times greater than the recommended concentration. A major focus of interest of these investigators was whether or not IMTX, an inert montmorillonite clay-based adsorbent, at this dietary concentration would result in any nutrient deficiencies since some clay-based adsorbent products, when supplemented to poultry diets, have been reported to impair nutrient utilization. The data collected in their study clearly showed that IMTX fed at 1.0% of the diet was able to totally remove the negative effects in broilers of feeding 4 mg/kg aflatoxin B_1 and did not result in any vitamin, mineral or other nutrient deficiencies compared to control broilers receiving no IMTX in their diet.

The most costly component of a poultry diet is metabolizable energy. In certain parts of the world, for several reasons, the poultry industry often uses diets containing lower than the desired concentration of metabolizable energy. In many cases, this is a result of having to formulate using feed ingredients of poor quality. In other instances, the luxury of having high energy ingredients available for feed formulation may not exist. Since the safety of IMTX, has not been tested in broiler diets formulated to have variable concentrations of metabolizable energy, Milwhite, Inc. decided to have this type of research conducted. In these experiments, it was decided to supplement up to 2.0% IMTX which was 8 times the recommended concentration and twice the quantity used in the safety study reported by Ledoux et al. (1999). Indeed, if IMTX was binding any vitamins or minerals thus, making them unavailable to the bird then a negative effect would be expected in broiler performance, especially in the diets containing low concentrations of metabolizable energy.

RESULTS AND DISCUSSION

Mortality during the above study was less than 1% and not related to dietary treatment. As would be expected in an experiment with this design, increasing the amount of energy in the diet increased body weight and improved feed conversion at 7, 14 and 21 days. IMTX had no influence on body weight or feed conversion at any energy level at any age. When the metabolizable energy was increased from 2,940 to 3,100 kcal ME/kg diet the excreta moisture increased from 60.4 to 65.8% and excreta moisture was not affected by the addition of IMTX to the diet. However, there was a decreasing trend in excreta moisture, which averaged 64.2, 62.9 and 62.1% for birds fed 0, 1.0 and 2.0% IMTX, respectively.

In summary, feeding up to 8 times the recommend dietary concentration of IMTX did not cause any negative effects on feed consumption, body weight, feed conversion, or excreta moisture of broilers fed diets varying in their concentration of metabolizable energy up to 3 weeks of age.

Note: A complete description of the experiment conducted at the University of Florida and the data collected in the experiment can be found in the referenced publication (Miles and Henry, 2007) located in the footnote below. The information presented in this issue of MILWHITE'S INFORMATION PAGE was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Miles, R.D. and P.R. Henry. 2007. Safety of Improved Milbond-TX® when fed in broiler diets limiting in available phosphorus or containing variable levels of metabolizable energy. Journal of Applied Poultry Research. 16:412-419.
Ledoux, D.R., G.E. Rottinghaus, A.J. Bermudez and M. Alonso-Debolt. 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poultry Science. 78:204-210.

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MILWHITE, INC.
JOURNAL

JANUARY/2015

IMPROVED MILBOND-TX®

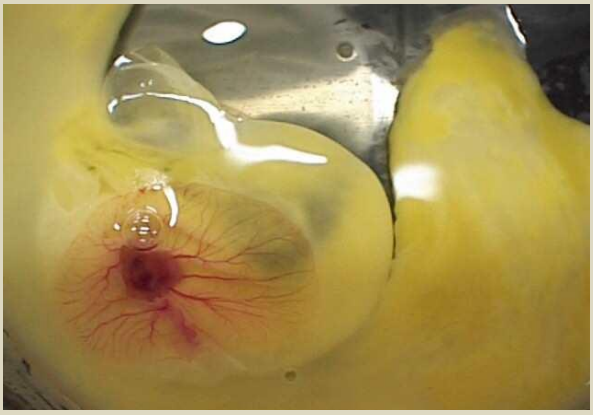
SAFETY STUDIES WITH BROILER BREEDERS

Data collected in previous studies with broilers and laying hens have documented the safety of Improved Milbond-TX® (IMTX) when supplemented at or above the recommended dietary concentration of 0.25% (2.5 g/kg diet). The summarized results of these studies have been the focus of previous issues of **Milwhite's Journal**. Even though IMTX has successfully been used for many years at the recommended concentration in broiler breeder diets without any detrimental effects being reported by the poultry industry, no safety studies have been conducted to ensure that higher than recommended concentrations are safe for breeders. The data collected in these studies would be of extreme importance since breeder birds are not only consuming nutrients and energy required for their own maintenance and well-being, the hen is also producing eggs which must also contain adequate nutrients and energy to insure proper embryo development and survival. If IMTX was responsible for binding any one or more nutrients in the diet and limiting nutrient absorption by the hen then some aspect of production and chick development should be noticed. Therefore, in 2013 at the University of Arkansas Center of Excellence for Poultry Science, Dr. Craig Coon initiated a study specifically designed to investigate the safety of IMTX when added to diets of broiler breeders at higher than the recommended amount.

The Cobb 500 broiler breeder pullets and MX males used in this study were fed a diet adequate in all nutrients and energy on a skip-a-day feeding schedule during their growing period. The pullets were fed an amount of feed required for them to attain the body weight necessary to maintain their weight according to the growth curve recommended by the primary breeder. Males were also fed according to the recommendations of the primary breeder. At 21-weeks of age, birds were allocated randomly to 12 floor pens with four pens per dietary treatment each containing 25 hens and 2 roosters, switched to a breeder diet and light stimulated. The experimental breeder diets were then offered to each pen of birds with each pen receiving the same quantity of feed/bird daily up to peak egg production and the quantity of feed adjusted for any mortality. Experimental treatments consisted of a corn-soybean meal-based control diet (0% IMTX) and two other diets containing IMTX supplemented at twice the recommended amount (0.5%) and four times the recommended amount (1.0%), respectively. The diets contained 15.5% crude protein and 2915 kcal AME/kg diet and were formulated on an ideal protein basis using digestible amino acids. At peak egg production each hen was consuming 450 kcal of AME and 23.9 grams of crude protein in 154 grams of feed. Following peak egg production feed allocation gradually declined to quantities necessary to maintain egg mass and body weight.

Egg collection began at week 24 and three times each day all eggs laid were collected and marked by pen number during the entire 12-week experimental period. All eggs collected each week were set in an incubator for hatching.

CHICKEN EMBRYO



Egg weights were determined on all eggs laid for two days during each week. At egg transfer, on day 18 of incubation, eggs were candled to determine those that were infertile and early embryo mortality. At hatch, each chick was examined visually for any abnormality and weighed individually using a digital scale.

Results: After the data collected during the study were analyzed statistically, it was found that supplementing IMTX at a higher dietary concentration than recommended did not have any effect on egg production expressed as eggs per hen housed. In fact, the value for egg production was numerically higher in hens consuming the diets containing IMTX. The number of eggs per hen housed during the entire experimental period for the three dietary treatments of 0%, 0.5% and 1.0% IMTX was 55.5, 57.8 and 56.9, respectively.

Egg weights, fertility, hatchability and chick weights were not affected by the addition of IMTX to the diet of broiler breeders. At the end of the 12-week experimental period egg weights for the dietary treatments of 0%, 0.5% and 1.0% IMTX were 62.8, 62.2 and 62.2 grams, respectively. Percent egg fertility was 98.4, 97.5 and 98.6% for the three dietary treatments, respectively, and percent hatchability of all eggs set was 89.0, 87.4 and 87.7%, respectively, for the three dietary treatments. Chick weights were not significantly affected at any week throughout the entire 12 week experimental period by the addition of the high dietary levels of IMTX. Averaging chick weight for the experimental period for the three dietary treatments was 40.1, 40.1 and 40.4 grams, respectively.

In summary, feeding up to 4 times the recommended dietary concentration of IMTX for 12 consecutive weeks, beginning at first egg, did not cause any negative effects on any aspect of broiler breeder performance.

*Note: A complete description of the experiment conducted at the University of Arkansas and the data collected in the experiment can be found in the referenced publication (to be written) located in the footnote below. The information presented in this issue of **Milwhite's Journal** was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL,USA*

Schlumbohm, M.J., England, J.A., Kriseldi, R., and Coon, C.N. Safety of Improved Milbond-TX® mycotoxin binder when fed to broiler breeders above the recommended levels. International Journal of Poultry Science 13 (10): 597-601, 2014.

MILWHITE, INC.
JOURNAL
FEBRUARY/2015

IMPROVED MILBOND-TX®

"SAFETY STUDY WITH LOW PHOSPHORUS LEVELS"

There are several reports in the scientific literature indicating that some clay-based adsorbents will interfere with an animal's ability to utilize nutrients. Also, it is a well-known fact among animal nutritionists that aluminum (Al) will complex with P in the digestive tract causing decreased bone mineralization and increased bone reabsorption. Ledoux et al. (1999) was able to demonstrate that when 1.0% Improved Milbond-TX® (IMTX) was supplemented to broiler diets nutrient utilization was not impaired when performance of birds fed IMTX was compared to control birds fed diets containing no IMTX. Miles and Henry (2007a) fed up to 2.0% IMTX to broilers in a study designed to specifically determine its safety at high levels of dietary inclusion. Even at a dietary concentration which was twice the amount included in the diet by Ledoux et al. (1999) no indication of impaired nutrient utilization was reported. In their study, Miles and Henry (2007a) also reported that serum P concentration and percent bone ash (as measured by toe ash) were not affected by IMTX. A normal serum P concentration in broilers was also documented in the publication of Ledoux et al. (1999) when IMTX was fed at a dietary concentration of 1.0%. Therefore, it was concluded by these investigators that dietary P was not being sequestered by the aluminum (Al) in IMTX since IMTX contains between 100 to 136 gm Al/kg as Al_2O_3.

Even though Ledoux et al. (1999) and Miles and Henry (2007a) were able to show that feeding high dietary concentrations of IMTX in diets adequate in all nutrients (including P) and metabolizable energy did not inhibit P utilization, the question arose: would IMTX have a negative effect on overall bird performance and bone mineralization if it were fed to broilers at high dietary concentrations in diets containing low P. This would be extremely important to know since less inorganic P is used in formulating poultry diets when phytase is used to enhance the utilization of the organically bound phytate P. Thus, a study was initiated at the University of Florida, using diets containing low P, in order to determine if supplementing high concentrations of IMTX would indeed bind P and decrease its utilization as reflected in poor overall broiler performance and/or decreased bone ash.

In this low phosphorus safety study, 450 Ross X Ross male broilers were selected from a larger group of chicks, weighed and allotted randomly with 5 birds per pen, 10 pens per treatment, in Petersime battery brooders. Each pen contained approximately the same total weight of chicks to eliminate extremes in initial body weight variability. IMTX was added at 0, 1.0 and 2.0% to each of 3 corn-soybean meal diets formulated to be isocaloric and isonitrogenous and contain 0.22, 0.32 or 0.42% available P. The diets and water were available *ad-libitum* throughout the entire 21-day experimental period. All birds and feed were weighed every 7 days and average treatment body weight, feed consumption and feed conversion were summarized.

Percent toe ash was selected as a measure of bone mineralization since it is a well-established method of determining bone mineralization rather than tibia ash because of the simplicity of toe collection and preparation compared with that required for bone ash. Hence, no ether extraction or removal of flesh tissue is required when using toe ash as the measure of bone mineralization. At the end of the 21 day experimental period for each experiment all chicks were killed and the middle toes of each chick were removed at the tarsometatarsal/P3 joint with the skin intact. The toe tip containing the nail was removed and discarded, then each toe was cleaned of any adhering foreign material with a wet paper towel and pooled by pen for toe ash analysis. Each set of toes was dried in an oven for 48 hours at 100°C, then ashed in a muffle furnace at 550°C for 14 hours to determine percent toe ash.

Results indicated that the effect of P in this study was highly significant. This meant that in the control and IMTX treatments when P in the diet increased feed consumption and body weight of chicks also increased. Feed conversion also improved as dietary P increased. There was no effect on body weight, feed consumption or feed conversion from feeding IMTX at 1.0 or 2.0% of the diet at any dietary P concentration. Toe ash was significantly lower when available P was deficient in the diet but was not affected by the addition of IMTX at any dietary P concentration. Even though, IMTX contains Al in its structural matrix, the data collected in this study clearly validate the fact that the Al in IMTX is not free to bind P in the digestive tract. Thus, if a feed mixing error occurs and IMTX is added to poultry diets at up to 8 times the recommended level no detrimental effects on bird performance should be expected (Miles and Henry, 2007b).

Note: A complete description of this experiment conducted at the University of Florida and the data collected in the experiment can be found in the referenced publication (Miles and Henry, 2007b) located in the footnote below. The information presented in this issue of MILWHITE'S INFORMATION PAGE was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Miles, R.D. and P.R. Henry. 2007a. Safety of Improved Milbond-TX® when fed in broiler diets at greater than recommend levels. Animal Feed Science and Technology. 138:309-317.

Miles, R.D. and P.R. Henry. 2007b. Safety of Improved Milbond-TX® when fed in broiler diets limiting in available phosphorus or containing variable levels of metabolizable energy. Journal of Applied Poultry Research. 16:412-419.

Ledoux, D.R., G.E. Rottinghaus, A.J. Bermudez and M. Alonso-Debolt. 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poultry Science 78:204-210.

MILWHITE INC.
JOURNAL

MARCH 2015

IMPROVED MILBOND-TX®

"*IN-VIVO* AFLATOXIN B_1 BINDING IN PIGS"

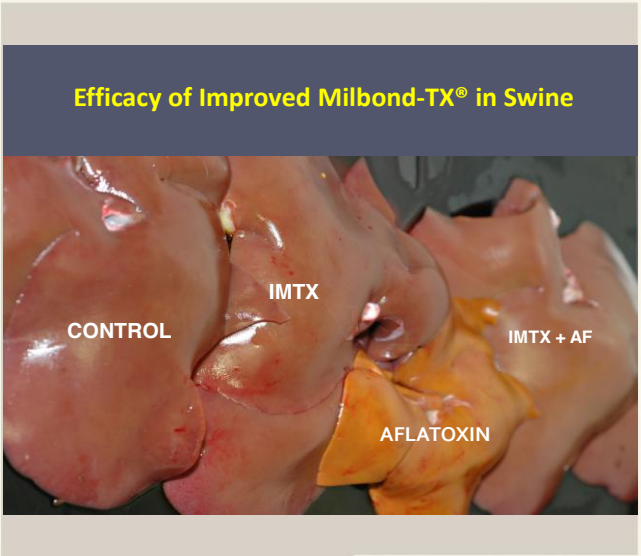
The research data reported in this issue of Milwhite's Journal is a summary of the data taken from a final report from a swine study conducted by Dr. David Ledoux and his research team at the University of Missouri at the request of Milwhite, Inc. This research with pigs was conducted by Dr. Ledoux following the publication of his *in-vitro* and subsequent *in-vivo* research using commercial broilers which showed that adding 1% Improved Milbond TX® (IMTX) to a diet containing 4 ppm AFB_1 was safe and totally effective in preventing the toxic effects on broiler performance that are commonly associated with aflatoxicosis (Ledoux et al., 1999). The objectives of this research with pigs were to determine the ability of IMTX to effectively prevent the toxic effects associated with AFB_1 when added to a swine diet and to demonstrate that the addition of IMTX to the swine diet was safe and would not have a negative effect on swine performance.

EXPERIMENTAL DESIGN AND DATA COLLECTED

In this study, 6 weanling pigs were assigned to each of 4 dietary treatments beginning 14 days after the pigs were weaned. Each pig was weighed individually on day 0 (14 days before weaning), day 14 (after weaning) and on days 21, 28 and 35. Dietary treatments were: 1) basal corn-soybean meal diet (no AFB_1 or IMTX); 2) basal diet + 0.5% IMTX; 3) basal diet + 3 ppm AFB_1; and 4) basal diet + 0.5% IMTX + 3 ppm AFB_1. During the entire 21-day experimental feeding period (days 14 to 35) all pigs had ad-libitum access to their experimental diets and water. During the experimental period feed consumption was recorded and feed conversion was calculated each 7 days. At the end of the experimental period (day 35) each pig was bled by jugular puncture and serum chemistry profile components determined (i.e., glucose, urea N, Ca, P, Mg, Na, K, Cl, creatine). Following blood collection each pig was killed by captive bolt head puncture followed by exsanguination and their liver and kidney removed, weighed and samples of each collected for histological examination. Also, samples of each section of the small intestine were taken from each pig for morphological evaluation.

RESULTS

During the 21-day experimental period pigs fed the diet containing 0.5% IMTX alone had similar feed intakes, body weights and grew as efficiently as those pigs fed the basal diet alone. Also, IMTX fed alone did not have any negative effect on liver and kidney weights. However, when AFB_1 was added to the basal diet pigs ate 57% less feed, gained 87% less body weight and had poorer feed conversion, all of which were significantly different ($P < 0.05$) from pigs fed the basal diet alone. When IMTX was added to the diet containing 3 ppm AFB_1 all of the growth depressing effects caused by the AFB_1 were not observed in the pigs.
The serum chemistry profile in pigs fed IMTX alone was similar to that of the pigs fed the basal diet alone. It is well established that aflatoxicosis is associated with the increase in two key enzymes in the serum of animals. These two enzymes are aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) and are important in normal liver function. During liver damage these enzymes are released from the liver and enter the serum. Also, aflatoxicosis is associated with high serum levels of direct bilirubin (DBili) and total bilirubin (TBili), thus indicating liver damage. DBili and TBili are by-products of the breakdown of the red blood cell. In this study, the addition of AFB_1 alone to the diet of pigs resulted in an increase ($P < 0.05$) in serum AST and GGT as well as an increase in serum DBili and TBili ($P < 0.05$). Adding IMTX to the diet containing AFB_1 resulted in similar levels of AST, DBili and TBili as observed in the serum of pigs fed the basal diet alone. The GGT level in the serum resulting from the addition of IMTX to the diet containing AFB_1 was significantly lower ($P < 0.05$) than that of pigs fed AFB_1 alone, but did not decrease to the level observed in the serum of pigs fed the basal diet alone.



Compared to the pigs fed the basal diet, adding AFB_1 alone to the diet significantly ($P < 0.05$) decreased the mucosal thickness in all three sections of the small intestine. The addition of IMTX to the diet containing AFB_1 restored the thickness of the mucosa in all three sections. Also, AFB_1 had a negative effect on the height of the villi in the small intestine, thus decreasing villus height in the duodenum, jejunum and ileum. A reduced crypt depth in the ileum and jejunum as well as a reduced villus height to crypt depth ratio in the duodenum was also attributed to feeding AFB_1. When IMTX was added to the diet containing AFB_1 the microscopic anatomy of the small intestine was totally restored and was similar to that of pigs fed the basal diet alone.

CONCLUSIONS

In this study, the addition of 0.5% IMTX to a pig diet based on corn and soybean meal resulted in normal pig performance. Supplementing 3 ppm AFB_1 alone to the basal diet resulted in a significant depression in pig performance. When IMTX was added to the diet containing AFB_1 alone body weight gain, feed intake and feed conversion were restored and were equal to that of pigs fed the basal diet containing no AFB_1. The binding and elimination of AFB_1 from the digestive tract by IMTX also resulted in restoring normal organ weights and prevented liver and kidney damage. No histological damage to tissue, no decrease in serum chemistry profile components and no observable nutritional deficiencies resulted from adding IMTX alone to the diet. In summary, the data collected in this *in-vivo* study with pigs indicated that feeding 0.5% IMTX alone and in a diet containing up to 3 ppm AFB_1 is safe and is effective in preventing the toxic and destructive effects on pig performance that are commonly associated with aflatoxicosis.

Note: The information presented in this issue of Milwhite's Journal was compiled from a final research report submitted by Dr. David Ledoux to Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. This issue of Milwhite's Journal was compiled by Dr. Orlando Osuna and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Ledoux, D.R., G.E. Rottinghaus, A.J. Bermudez and M. Alonso-Debolt. 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poultry Science. 78:204-210.

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MILWHITE INC.
JOURNAL

APRIL 2015

IMPROVED MILBOND-TX®

"SAFETY STUDY DEMONSTRATING NO BUFFERING CAPACITY"

In the young pig at weaning, as well as in other animals, it is desirable to maintain a low stomach pH. A pH of 4.0 or lower activates the enzyme, pepsin, which is required for the initiation of protein digestion associated with the unfolding of the natural protein structure in feed ingredients of plant and animal origin. A low pH also promotes the release of minerals and some vitamins which are bound to proteins. Mineral dissociation (solubilization) from compounds such as $CaCO_3$, $FeSO_4$ and $MgCO_3$ occurs at a low pH and allows Ca, Fe, and Mg, as well as other minerals, to be available for absorption. The enzyme, phytase, which liberates organically bound P for absorption, is most active at a low pH. Of equal importance, a low pH promotes overall animal health by inhibiting the growth of pathogenic bacteria such as *Salmonella, Streptococci, E. coli*, *Staphylococcus and Pseudomonas*. The ideal pH promoting growth of these pathogens is specific for each pathogen, but in the digestive tract a higher pH encourages multiplication. The ideal pH range for these bacteria is between 6 and 8, however, at a pH of 4.0 and below these pathogens do not proliferate. In order to maintain a low pH adequate hydrochloric acid (HCL) must be produced at a rapid rate by specialized stomach cells. This is especially true following a meal when the stomach pH increases due to the buffering capacity of various feedstuffs used in diet formulation. Unfortunately, the young pig's stomach is not fully developed and has a limited ability to produce HCL. Because of this, any feed additive that would have a tendency to raise the pH by neutralizing the HCL would not be a desirable component of the feed. This would also be of concern for the newly hatched chick since its digestive tract is also immature for several weeks following hatch.

In over 30 *in-vitro* and *in-vivo* studies conducted during the past 25 years, Improved Milbond-TX® (IMTX) has been tested for its safety and efficacy. A recent *in-vitro* study conducted in the laboratory of Dr. John Driver in the Department of Animal Sciences at the University of Florida provided further evidence showing IMTX, when used at the recommended dietary concentration of 0.25% (2.5 g/kg diet), is inert and has no ability to neutralize a solution of HCL at a pH of either 2.0 or 5.0. This finding has been of extreme interest to swine producers using IMTX in their baby pig diets. The following describes the *in-vitro* experiment.

At weaning, a pig will normally weigh between 5 and 6.5 kilograms and will be consuming between 225-325 grams of feed each day. Soon after being weaned feed consumption increases quickly. In order to determine the amount of IMTX to use in the experiment, the total amount in 225 gm of feed was used in the calculation. This amounted to 0.56 gm of IMTX (225 gm X 0.0025 = 0.56 gm). Also, the amount of IMTX in one pound of feed (454 grams) when used at the recommended concentration of 0.25% was chosen instead of 325 grams in order to give IMTX more opportunity to buffer the HCL solution, if indeed, it had any buffering capacity. This amounted to 1.1 grams of IMTX (454 gm X 0.0025 = 1.1 gm) which would be added to the un-buffered solutions of HCL already containing the 0.56 grams of IMTX. In so doing, this extra concentration of IMTX added to the solution already containing 0.56 grams of IMTX would provide the presence of even more IMTX to buffer the HCL solution. The two HCL solutions with a pH of approximately 2.0 and 5.0 were made by adding HCL to 200 ml of deionized water and checked with a pH meter. The above amounts of IMTX were added to each HCL solution and following 30 seconds of mild stirring the pH was immediately recorded.

RESULTS

(Note: Prior to adding the first quantity of IMTX to the HCL solutions with a pH of 2.0 and 5.0, the pH of each solution was checked and recorded as 2.05 and 4.98, respectively).

Low pH solution: Thirty seconds after adding 0.56 grams of IMTX to the HCL solution with a pH of 2.05 the pH was 2.06. Thirty seconds after adding the 1.1 grams of IMTX to this solution the pH was 2.10. So, after adding a total of 1.66 grams of IMTX to this HCL solution with an initial pH of 2.05 the pH only increased by 0.05 pH units. Thus, the 1.66 grams of IMTX added to the HCL solution reflected a dietary IMTX concentration of 0.36% instead of the recommended 0.25%.

High pH solution: Thirty seconds after adding 0.56 grams of IMTX to the HCL solution with a pH of 4.98 the pH was 5.01. Thirty seconds after adding the 1.1 grams of IMTX to this solution the pH was 5.05. So, after adding a total of 1.66 grams of IMTX to this HCL solution with an initial pH of 4.98 the pH only increased by 0.07 units. As above, this represented a dietary IMTX concentration of 0.36% instead of the recommended 0.25%.

CONCLUSIONS

These data collected in this experiment provide conclusive evidence that when IMTX is added to a weaned pig's diet at the recommended dietary concentration would not be expected to have any measurable negative effect on the pH of the fluid contents in the stomach. This would be true even if IMTX was accidently added to a diet at a slightly higher quantity than the recommended concentration of 0.25%. Furthermore, it must be kept in mind that pigs consume their feed all throughout the day and in-so-doing would not consume at any one feeding the total amount of IMTX used in this in-vitro experiment. Therefore, adding IMTX at the recommended dietary concentration of 0.25% is safe and has no buffering capacity which would tend to neutralize the pH in the stomach of a pig or other animal.

Note: This *in-vitro* experiment is unpublished and was conducted in order to answer the question from swine producers about the buffering capacity of IMTX, especially in weaned pigs. The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

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IMPROVED MILBOND-TX®

"IN-VITRO AFLATOXIN B_1 BINDING CAPABILITY"

Milwhite, Inc. introduced Improved Milbond-TX® to the animal and feed industry as a mycotoxin binder in 1992. Since then it has proven to be an efficient and safe product that continues to gain acceptance by the industry and provide assurance that when animals are fed diets containing aflatoxin (AF), IMTX has the ability to prevent the toxic effects associated with this mycotoxin. It is vital, when selecting any mycotoxin binder which is intended to be used in the feed, to demand to see both *in-vitro* and *in-vivo* data with regards to the ability to bind a specific mycotoxin. No mycotoxin binder should ever be purchased if it has not been tested under "conditions of actual use" which is in the animal. The reason for this is because some mycotoxin binders that have been tested have the ability to bind 100% of a specific mycotoxin in the test tube, but in the animal the same binder has 0% binding capability. This does not mean that *in-vitro* testing is unimportant. So, one might ask: Why go to all the trouble of testing a binder *in-vitro* if *in-vivo* testing is much more important? The reason is because *in-vitro* testing is a very good laboratory tool to use in order to screen different mycotoxin binders. Thus, a high ability to bind a specific mycotoxin in the test tube would open the window for further testing as an effective mycotoxin binder in the animal. Also, *in-vivo* testing is much more expensive and time consuming than *in-vitro* testing and separating out the binding potential of a product by first conducting *in-vitro* screening saves a lot of time, effort and cost.

During its development, IMTX was tested extensively "in-house" by Milwhite, Inc. in their laboratory. When convinced it had promise as a binder, Dr. David Ledoux at the University of Missouri (USA) was contacted to further test IMTX because his laboratory has the ability to test a mycotoxin binder *in-vitro* and *in-vivo*. Also, being tested independently in well-designed and controlled experiments would provide consumers with more confidence and assurance that IMTX is a safe and effective mycotoxin binder. The information in this issue of Milwhite's Journal briefly summarizes the *in-vitro* research that Dr. Ledoux conducted prior to his testing of IMTX *in-vivo* using broilers. A complete and detailed description of the materials and methods used in this *in-vitro* testing of IMTX to bind AFB_1 can be found in the publication of this research (Ledoux et al., 1999).

In his laboratory and working cooperatively with the Veterinary Medical Diagnostic Laboratory at the University of Missouri, Dr. Ledoux purchased purified Aflatoxin B_1 (AFB_1) from Sigma Chemical Company. The binding ability of IMTX was tested in four solutions with each solution having a different pH. This was done because in the animal's digestive tract the pH can range from very acid to basic as material moves from the anterior to the posterior of the tract. IMTX was tested for its ability to bind AFB_1 in 50 ml of a buffered solution at pH of 3, 5, 7 and 9 each of which contained 2 micrograms of AFB_1 per ml. To each of these pH solutions containing AFB_1, 0.5 grams of IMTX was added and stirred continuously for 30 minutes. Samples of these solutions were then tested for AFB_1 using HPLC.

RESULTS

The ability of IMTX to bind AFB_1 in each of the above mentioned solutions at pH of 3, 5, 7 and 9 was found to be 100%. Furthermore, in subsequent *in-vitro* testing at a pH of 3 to 9, IMTX was also shown to be able to bind 100% of the AFB_1 in solutions containing 250, 500 and 1,250 micrograms of AFB_1. The data collected in this laboratory experiment with IMTX provided the needed evidence showing that IMTX was, indeed, a prime candidate for further *in-vivo* research using the broiler as the experimental animal. The *in-vivo* research conducted by Dr. David Ledoux and his colleagues at the University of Missouri documenting the ability of IMTX to bind AFB_1 will be the focus of the next issue of **Milwhite's Journal.**

*Note: A complete description of the in-vitro experiment conducted at the University of Missouri and the data collected in the experiment can be found in the referenced publication located in the footnote below. The information presented in this issue of **Milwhite's Journal** was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.*

Ledoux, D.R., G.E. Rottinghaus, A.J. Bermudez and M. Alonso-Debolt. 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poultry Science. 78:204-210.

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MILWHITE INC.
JOURNAL

JUNE 2015

IMPROVED MILBOND-TX®

"IN-VIVO AFLATOXIN B_1 BINDING CAPABILITY"

In the previous issue of Milwhite's Journal the *in-vitro* aflatoxin B_1 (AFB_1) binding ability of Improved Milbond TX® (IMTX) was discussed. This *in-vitro* research was conducted by Dr. David Ledoux and his research team at the University of Missouri. The data reported showed that IMTX had great potential as a mycotoxin binder in the animal since it had extremely high *in-vitro* binding ability. Their *in-vitro* data showed that in solutions with pH of 3, 5, 7 and 9, IMTX was able to bind 100% of the AFB_1 concentration added to each solution. Following the *in-vitro* study Dr. Ledoux and his team conducted an *in-vivo* study with commercial broilers. This present issue of Milwhite's Journal discusses the *in-vivo* data collected with broilers showing that adding 1% IMTX to the diet was able to efficiently bind AFB_1 in the digestive tract so that all of the negative effects associated with feeding 4 ppm AFB_1 to the broiler were eliminated and broiler performance was totally restored.

EXPERIMENTAL DESIGN AND DATA COLLECTED

A total of 120 day-old male broilers were weighed, wing-banded and assigned to pens in stainless steel chick batteries. There were 5 replicate pens of 6 chicks each in each of 4 dietary treatments offered *ad-libitum* throughout the entire 21 day experimental period. Treatments were: 1) basal corn-soybean meal diet (no AFB_1 or IMTX); 2) basal diet + 1% IMTX; 3) basal diet + 4 ppm AFB_1; and 4) basal diet + 1% IMTX + 4 ppm AFB_1. On day 21, 10 chicks from each treatment (2/pen) were bled by cardiac puncture and serum chemistry profiles determined. Also, 15 chicks from each treatment (3/pen) were killed by cervical dislocation and various organs weighed and tissue samples taken and processed for histological examination. All birds were examined for gross pathological signs due to feeding the AFB_1 or resulting from possible nutritional deficiencies due to consuming the IMTX.

RESULTS

Only 5 chicks died during the entire 21-day experimental period and the researchers did not attribute these deaths directly to any particular dietary treatment. The average body weight and feed intake of chicks consuming the basal diet supplemented with 1% IMTX were no different than the body weight and feed intake of chicks consuming the basal diet. However, average body weight and feed intake of chicks fed the basal diet containing 4 ppm AFB_1 were significantly less ($P < 0.0001$) than those chicks fed the un-supplemented basal diet. When IMTX was supplemented to the diet containing 4 ppm AFB_1 the average chick body weight and feed consumption were totally restored and chick performance was numerically better than that of chicks fed the basal diet alone. Data showed that chicks fed the combination AFB_1/IMTX diet vs basal diet consumed similar amounts of feed (1000 vs 964 g), grew as well (796 vs 779 g) and were as efficient in feed conversion (1.26 vs 1.24 g:g). Feeding AFB_1 alone resulted in heavier organ weights (liver, heart, kidney, proventriculus, and pancreas) compared to those organs from chicks fed the basal diet alone. The livers of chicks fed AFB_1 alone were pale and enlarged with rounded margins. Chicks fed the diet containing IMTX alone and the IMTX/AFB_1 diet had organ weights similar to those of chicks fed the basal diet alone. In this study, the average weights of the spleen, bursa of Fabricius and gizzard were not affected by any dietary treatment. Feeding AFB_1 alone decreased ($P < 0.05$) the serum chemistry profile values (i.e., calcium, phosphorus, cholesterol, glucose, albumin, total protein and globulin) compared to the profile values of chicks fed the basal diet and the IMTX diet alone. When IMTX was added to the diet containing AFB_1, with the exception of glucose and cholesterol, all of the other serum chemistry profile values were restored to those of chicks fed the basal diet.

Upon gross examination of the chick organs no nutritional related deficiencies could be found in any of the organs from chicks fed any of the treatments. With the exception of liver and kidney, in general, there were no obvious microscopic lesions in any of the other tissues. Chicks fed AFB_1 alone had moderate to severe fatty infiltration of the liver and significant kidney pathology with thickening of the glomerular capillary basement membrane. No kidney pathology was observed in chicks fed the AFB_1/IMTX diet.

CONCLUSIONS

Compared to the body weight and feed intake of broiler chicks fed a corn-soybean meal basal diet, adding 4 ppm AFB_1 to the diet resulted in a 25% decrease in body weight, a 22% decrease in feed intake, increased organ weights and liver and kidney damage as evidenced by histological examination. Adding 1% IMTX to the diet containing the AFB_1 completely restored chick body weight and feed intake. This demonstrated the successful ability of 1% IMTX to bind up to 4 ppm AFB_1 in the digestive tract of broilers, thus eliminating all of the negative effects that AFB_1 was shown to have on broiler performance. The binding and elimination of AFB_1 from the digestive tract by IMTX also resulted in restoring normal organ weights, normal serum chemistry profiles and prevented liver and kidney damage. No histological damage to tissues and no observable nutritional deficiencies resulted from adding IMTX alone to the diet. In summary, the data collected in this 21-day *in-vivo* study with broiler chicks indicated that feeding 1% IMTX alone and in a diet containing up to 4 ppm AFB_1 is safe and is effective in preventing the toxic effects on broiler performance that are commonly associated with aflatoxicosis.

Note: A complete description of the in-vitro and in-vivo experiments conducted at the University of Missouri and the data collected in the experiments can be found in the referenced publication located in the footnote below. The information presented in this issue of ***Milwhite's Journal*** *was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.*

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JOURNAL

JULY 2015

IMPROVED MILBOND-TX®

"REDUCTION OF AFLATOXIN TRANSFER FROM FEED TO MILK"

TABLE 1. PERCENT *IN-VITRO* AFLATOXIN B_1 BOUND BY 8 DIFFERENT ADSORBENTS*

ADSORBENT NAME	PERCENT AFB_1 BOUND	COEFFICIENT OF VARIATION
MTB-100	43.43	2.28
ULTRASORB	98.80	1.83
MEXSIL	97.48	1.28
CONDITION ADE	99.51	8.97
NOVASILPLUS	99.45	4.00
TOXYNIL+	99.31	6.91
ASTRAN BEN 20 A	96.17	4.89
MILBOND-TX	98.21	2.10

*DATA TAKEN FROM STROUD et al., 2006

TABLE 2. PERCENT REDUCTION IN MILK AFLATOXIN (AFM_1) CONCENTRATION, SECRETION, AND TRANSFER FROM FEED TO MILK BY DIFFERENT ADSORBENTS ADDED TO DAIRY COW DIETS AT 0.5% OF THE DRY MATTER[1]

	...% REDUCTION IN...		
ADSORBENT NAME	MILK AFM_1 CONCENTRATION	MILK AFM_1 SECRETION	AFM_1 TRANSFER FROM FEED TO MILK
MTB-100	- 7.81	-6.71	-3.60
ULTRASORB	7.36	7.85	7.59
MEXSIL	6.62	8.00	7.19
CONDITION ADE	7.85	13.79	13.23
NOVASILPLUS	40.39*	42.59*	42.09*
TOXYNIL+	34.98*	36.36*	34.45*
ASTRAN BEN 20 A	48.90*	52.28*	48.44*
MILBOND-TX	46.49*	48.46*	44.55*
POOLED STD. ERROR	12.69	13.75	13.12

[1]DATA TAKEN FROM STROUD et al., 2006
*VALUES ARE DIFFERENT FROM ZERO WHEN P<0.05

If cows consume feed contaminated with the mold toxins of *Aspergillus flavus* and *Aspergillus parasiticus*, there are four sub-types of aflatoxin that may be consumed and these are Aflatoxin B_1, B_2, G_1 and G_2. Of these, Aflatoxin B_1 (AFB_1) is the most toxic and the sub-type that is most commonly detected in the feed. Once AFB_1 is consumed, absorbed from the digestive tract and enters the body it is then converted in the animal's liver into a metabolite known as AFM_1. In the case of a lactating cow, once produced, AFM_1 will then be secreted into the milk. Since aflatoxins, especially AFB_1, are some of the most carcinogenic substances that may possibly be consumed by animals, governments have set limits on the concentrations of aflatoxin in animal feed and milk. In the United States, for example, the Food and Drug Administration (FDA) has set aflatoxin limits in feeds consumed by dairy cows at 20 ppb. However, in milk intended for human consumption, the limit set by the FDA for AFM_1 is 0.5 ppb.

No mycotoxin binder should ever be recommended or purchased unless it has been tested for its effectiveness *in-vivo*. The reason for this is because an additive that has claims of being able to bind a specific mycotoxin may indeed be very effective and bind up to 100% of a specific mycotoxin *in-vitro*, but once added to the diet and consumed by an animal the same mycotoxin binder may have very little or no ability to bind the same mycotoxin. An excellent example of why an additive should be tested *in-vivo* is presented in Table 1. These data were taken from a research study conducted by Dr. Lon Whitlow at North Carolina State University and eventually published by Stroud et al., 2006. The research was conducted to determine the efficacy of 8 feed additives to reduce the transfer of aflatoxin from feed to milk.

EXPERIMENTAL TREATMENTS AND STUDY DESIGN

In this study, sixty lactating Holstein cows were randomly assigned to 9 dietary treatments. Eight adsorbents were each added at 0.5% of the dry matter to a total mixed ration and fed to dairy cows. From ration intake data, each cow consumed approximately 100 grams of adsorbent daily. The naturally contaminated corn used in the ration contained 800 ppb aflatoxin. Treatment replicates consisted of six cows fed each dietary treatment with a control group if 12 cows fed a diet containing no adsorbent. Following consumption of the experimental diets the milk was collected from each cow and tested for AFM_1. Further details and a complete description of the experimental methods used in this study can easily be found in the cited publication of Stroud et al. (2006).

RESULTS

It is clear from looking at the *in-vitro* AFB_1 binding data in Table 1 that seven of the adsorbents were able to bind more than 96% AFB_1 and one, MTB-100, was only able to bind 43.43%. IMPROVED MILBOND-TX (IMTX) was shown to have an ability to bind 98.21% of the AFB_1 used in the *in-vitro* test. This was not surprising, since similar results for IMTX have been reported in previous *in-vitro* AFB_1 binding tests conducted in numerous laboratories.

When looking at the *in-vivo* effectiveness of these 8 adsorbents to significantly reduce the total concentration of AFM_1 in milk, only four adsorbent products were successful and these were IMTX, NOVASILPLUS, TOXYNIL+ and ASTRA BEN 20 A.

Even though ULTRASORB, MEXSIL and CONDITION ADE were successful in binding above 97% AFB_1 *in-vitro*, these three adsorbents had minimal ability to decrease the total amount of AFM_1 in milk. Also, MTB-100 was the only additive, among the eight tested, that was shown to have poor AFB_1 binding properties both *in-vitro* and *in-vivo*. This additive was not a clay-based additive, but rather a product derived from yeast cell walls that contained non-digestible yeast oligosaccharides (i.e., glucomannans). On the other hand, it can also be seen in Table 2, when expressing their success in a different manner, the same 4 adsorbents that had the capability of significantly decreasing ($P < 0.05$) the total milk AFM_1 concentration had the highest percent reduction in milk aflatoxin secretion as well as the highest percent reduction in AFM_1 transfer from feed to milk.

The addition of mycotoxin binders to animal diets is now recognized as being one of the most effective approaches in preventing the absorption of mycotoxins across the animal's digestive tract. Once a mycotoxin is bound to an additive in the digestive tract it is only a short time before it is eliminated. The data collected in this study re-emphasize the importance of having a proposed dietary feed additive that is going to be used as a mycotoxin binder tested *in-vivo* using the animal species to which it will be fed. *In-vitro* testing should only be used to screen-out those products that have poor mycotoxin binding potential and identify those that have promise. Also, research with mycotoxin binders during the past 30 years has never been able to demonstrate that *in-vitro* results correlate well with *in-vivo* results. In other words, there is no *in-vitro* test presently available that is 100% reliable in predicting how effectively an additive will bind one or more mycotoxins in an animal's digestive tract. Therefore, continuous scrutiny of new products will assure that only the best additives will be available to the animal industry in order to minimize occurrences of mycotoxicosis in animals and prevent accumulation of mycotoxins in the products from these animals.

Note: This issue of Milwhite's Journal was compiled by Dr. Orlando Osuna and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Stroud, J.S. 2006. The Effect of Feed Additives on Aflatoxin in Milk of Dairy Cows Fed Aflatoxin-Contaminated Diets. M.S. Thesis. North Carolina State University, Raleigh, NC. USA

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MILWHITE INC.
JOURNAL

AUGUST 2015

IMPROVED MILBOND-TX®

"AFLATOXIN AND LIVER FLUKES IN DAIRY CALVES"

TABLE 1. ADDITIVE EFFECT OF AFLATOXIN B_1 (AFB_1) AND LIVER FLUKE INFESTATION IN DAIRY CALVES[1]

TREATMENT	CALF	LIVER FIBROSIS	NODULAR LIVER SURFACE	ECCHYMOTIC HEMORRHAGE	BILE DUCT THICKENING	LARGE GALLBLADDER	NUMBER OF FLUKES
METACERCARIAE ONLY	1	*	.....	***	**	.....	19
	2	*	*	.....	.....	.....	5
	3	**	.....	.....	*	.....	11
	4	*	.....	.....	*	*	17
METACERCARIAE + 0.5 MG AFB_1/KG BWT[2]	1	*	.....	.....	*	***	58
	2	*	.....	**	*	***	46
	3	**	.....	.....	**	*	39
	4	*	.....	.....	*	***	15
METACERCARIAE + 1.0 MG AFB_1/KG BWT[2]	1	**	**	**	.....	***	48
	2	**	**	.....	***	****	21
	3	***	***	***	**	*	95
	4	****	****	****	****	*	208

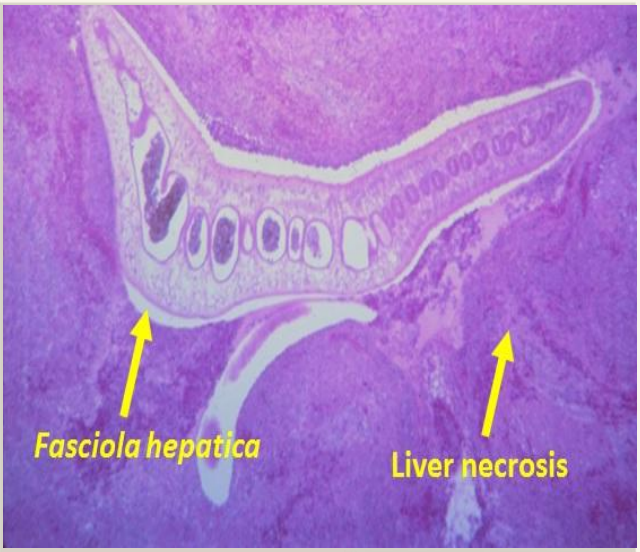
[1]ADAPTED FROM OSUNA ET.AL., 1977
[2]SINGLE ORAL DOSE OF AFB_1 GIVEN 5 WEEKS FOLLOWING METACERCARIAE INFESTATION IN THE 10 WEEK EXPERIMENT.
*, **, ***, **** = SEVERITY FROM MILD TO SEVERE

The liver must be maintained in a healthy state at all times because of the hundreds of vital roles it continuously plays each second in keeping the body healthy and functioning properly. Everything in an animal's diet that is consumed digested and absorbed will eventually pass through the liver. This is also true for ingested toxins and many parasites. In fact, even though there are other routes for toxins and parasites to enter the body, the oral route is the most common. A major liver function, that all liver cells have, is related to clearing the body of toxins and many times, in so doing, the liver may be damaged by the same toxins and their metabolites it is filtering, detoxifying and excreting. Once parasites find a home in the liver they continue to cause problems which will, in most cases, if not treated lead to death of the animal. Once damaged by a parasite such as the liver fluke (*Fasciola hepatica*) or by a toxin such as aflatoxin (AFB_1), any further insult to an already damaged liver will only make things worse. The worst-case-scenario would be for the liver to be forced to cope with AFB_1 after it has already been infested with *Fasciola hepatica* (Fh). The negative additive effects of the worst-case-scenario, just mentioned,was first reported in a publication by Osuna et al., (1977).

Since their discovery, only 55 years ago, aflatoxins have been the most intensively studied mycotoxins in the world. The research published by Osuna and his coworkers was conducted only 15 years from the time of the discovery of aflatoxins and was at a time when research in this area of toxicology was still in its infancy. Once it was discovered in 1961 that the mold responsible for producing aflatoxins was from the Genus *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus*, awareness about the detrimental effects that aflatoxins caused in animals increased rapidly throughout the world. Most of the early experiments regarding the influence that AFB_1 had on animal performance, after it was learned that AFB_1 was a hepatotoxin, used an experimental design that would explore the "dose response relationship" and "cause and effect" when AFB_1 was added to animal diets. Simply, different concentrations of AFB_1 would be fed and the effects in various animals would then be documented. However, the above research conducted by Osuna and coworkers in the middle 1970's was the first to show the synergistic effect that AFB_1 would have in young ruminants known to be previously infected with Fh. This research was of extreme importance at the time, since for thousands of years it had been known that Fh had a devastating effect on liver function, but nothing in the middle 1970's was known about the combined effect that the newly discovered AFB_1, a known hepatotoxin, and Fh would have when both were present simultaneously in the animal.

EXPERIMENTAL TREATMENTS AND STUDY DESIGN

The entire experimental design and the complete in-depth results of this study, especially with regards to the blood components, can be found in the cited publication. However, the following is only a summary of the design and major results observed upon biopsy and necropsy in this study. Three oral doses of AFB_1 (0.0, 0.5 and 1.0 mg/kg body weight) and 2 infection levels of Fh metacercariae (0 and 220) were used. (Note: metacercariae are the encysted resting or maturing stage of any trematode parasite and it is the infective stage of the trematode life cycle. Following ingestion, metacercariae hatch in the small intestine and migrate across the gut wall and to the liver where the juvenile flukes migrate through the liver tissue feeding and growing until they reach the bile ducts). Since young ruminants are more sensitive to AFB_1 than yearlings and adults, four male Holstein dairy calves weighing an average of 45.8 kg each were allocated randomly to each of six treatment groups. The single oral dose of 220 metacercariae was given to all animals, except the control, at the initiation of the 10 week experiment and five weeks later the single oral dose of AFB_1 was administered to all treatment groups except the control.



RESULTS

When compared to the control calves there were significant differences ($P<0.05$) in dry matter intake, body weight and serum albumin throughout the entire experimental period for treatment groups infected with metacercariae of Fh and AFB_1. The decrease in dry matter intake in the AFB_1 group of calves occurred immediately following the administration of the oral dose and the calves uninfected with metacercariae had a higher dry matter intake than infected calves. Once the oral dose of AFB_1 was administered an immediate decrease in body weight was observed.

Compared to the calves receiving only metacercariae (Table 1) the single dose of 0.5 mg AFB_1/kg body weight in the presence of metacercariae resulted in a definite increase in the number of Fh in the liver as well as an enlargement of the gallbladder. This observation was also apparent when the higher dose of AFB_1 was administered to the calves. When the overall observations from biopsies or necropsies presented in Table 1 were considered, calves infected with metacercariae and administered the highest concentration of AFB_1/kg body weight were the most seriously affected, thus, confirming the additive negative effects of AFB_1 and Fh.

In studying toxicology it should never be forgotten that the concentration of dose and duration of exposure have a pronounced effect on the toxicity of any toxin. This research, using one acute dose of AFB_1 in calves already infected with liver flukes contributed, at that time, to the "developing" base of knowledge about mycotoxins that had just been discovered 15 years earlier. These data collected in this publication and other data collected in the thousands of publications since this work was conducted has helped to understand the short and long term consequences of acute and chronic exposure to mycotoxins. Today, we know much more about AFB_1 and other mycotoxins than in the mid-1970's and today we also have inert clay-based feed additives, such as Improved Milbond-TX®, that are effective in binding AFB_1. Of course, the best way to protect animals from the negative effects of AFB_1 and other mycotoxins is to select and use feed ingredients in the diet that are of the highest quality.

Note: This issue of Milwhite's Journal was compiled by Dr. Orlando Osuna and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Osuna, O, G.T. Edds and H.D. Blankespoor. 1977. Toxic effects of aflatoxin B_1 in male Holstein calves with prior infection by flukes (*Fasciola hepatica*). Am. J. Vet. Res. Mar. 38(3) 341-349.

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MILWHITE INC.
JOURNAL

SEPTEMBER 2015

IMPROVED MILBOND-TX®
CLAY-BASED MYCOTOXIN BINDERS

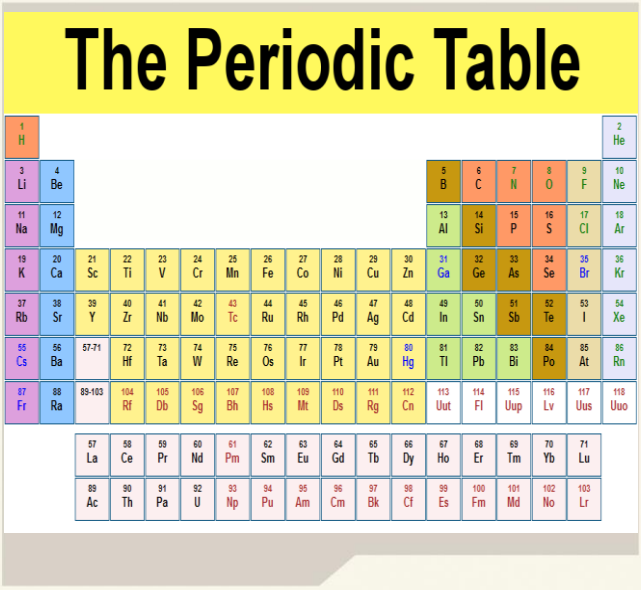
"UNDERSTANDING CLAYS: THEIR ORIGIN, FORMATION & LOCATION"

Improved Milbond-TX® (IMTX) is one of several inert clay-based mycotoxin binders sold commercially. Since the introduction of IMTX to the world-wide animal and feed industries in 1992, tens of thousands of tons have been successfully used as a dietary additive to prevent losses in animal performance commonly associated with aflatoxin B_1 (AFB_1). The question is often asked, "Why is IMTX such an effective binder of AFB_1?" It would be so easy to simply respond by saying, "The ability of IMTX to bind AFB_1 so effectively is due to the properties associated with a specific Family of clays called "Smectite" to which IMTX belongs." However, nothing would be learned by this simple answer. So, in this issue of Milwhite's Journal and in the next issue clays are discussed in a manner that promotes understanding and learning. This approach should provide enough background information about clays so that it will be easier to understand and appreciate the exact mechanisms involved when IMTX sequesters AFB_1 in an animal's digestive tract. The mechanisms involved in sequestering AFB_1 will be the topic of discussion in the next issue of Milwhite's Journal.

All clays consist of elements (atoms) combined with other elements to form compounds known as "minerals". Rocks are made of minerals and clays are formed when rocks are weathered. Simply speaking, the rocks that are eroded to form clays are combinations of different elements and there are 92 different elements associated with the earth's crust. Minerals have known compositions and unique crystalline structures. Because there are so many possible combinations of elements when they bind together geologists have discovered and classified over 4,000 minerals each with a different name, chemical composition and atomic structure. The same is true for clays, in that, as rocks erode into their basic mineral elements different clays form as these elements recombine or the parent mineral is dramatically altered. Therefore, the elements which comprise the mineral makeup of a clay and directly responsible for a clay's crystalline structure are a reflection of the elements in the parent mineral of a particular rock. Unless someone is a geologist by training, understanding the nomenclature and the elemental makeup of all the different minerals is very confusing and overwhelming to say the least.

Of the 92 elements in the earth's crust only 8 are found in large quantities and make up 98% of the crust. These elements are, in order of abundance, oxygen, silicon, aluminum, iron, calcium, sodium, potassium and magnesium. Oxygen, silicon and aluminum are the most abundant and these three alone make up approximately 85% of the earth's crust. The reason oxygen is so abundant is because as other elements combine with oxygen they form oxides. For example, aluminum bound to oxygen is known as alumina (Al_2O_3) and silicon bound to oxygen is known as silica (SiO_2) which is better known as "quartz". Crystals of SiO_2 can range in size from very large to very small as in "sand". It is unbelievable, but the largest quartz crystal ever to be discovered was approximately 20 feet long and weighed more than 48 tons. In nature, minerals are found in various degrees of purity. If one or more minerals are associated with a crystal of SiO_2 a completely different mineral is formed that has a different name with a different crystalline structure and different physical properties. Minerals containing silica are known as "silicates" and are the largest group of minerals. When minerals containing alumina and silica combine they are referred to as "aluminosilicates". In nature, the crystalline structure of aluminosilicate ores is usually associated with water. When this occurs the clays are referred to as "hydrated aluminosilicates" with a general formula of $Al_2O_3SiO_2H_2O$. When other elements such as sodium and calcium are involved with such a clay they are referred to as a hydrated sodium calcium aluminosilicate or better known simply as "HSCAS". It is because of the properties of HSCAS that they possess a high capacity to bind with AFB_1. Also, HSCAS clays ordinarily contain other minerals in small amounts. IMTX is classified as HSCAS clay.

Geologically speaking, clays are formed over millions of years in the earth's outer layer (i.e., crust) from the erosion of rocks, as previously mentioned. The eroded material (clay) can be found located in two places in reference to its parent rock. The clay deposit that required millions of years to form can be found very close to its parent rock or varying distances from its parent rock. The location of clay, in reference to its parent rock, gives rise to the name of the two main types of clay. Residual or "primary" clays, as they are sometimes called, are those clays that remain at or near their site of formation. Sedimentary or "secondary" clays are carried by wind, water or glaciers varying distances from where they are formed.



In fact, shale is the most abundant type of clay-rich sedimentary rock on earth and is found world-wide in sedimentary basins where it was deposited millions of years ago. Some of these basins are very large and give rise to many of the world's richest shale oil and natural gas deposits. These deposits were formed as a result of sedimentary clay material traveling over and covering lake beds and sea bottoms that were rich in algae. Then, after millions of years, as the algae and other organic material decomposed, formed the natural gas and shale oil deposits that are so valuable today.

As stated previously, IMTX is an inert clay-based material and there are indeed essential characteristics associated with certain clays, especially those belonging to the Smectite Family, which make them efficient mycotoxin binders. The Smectite Family consists of many different types of clay which are sometimes referred to as "clay species" within this Family. Smectite clays are known to possess two main properties that are responsible for them being efficient binders of AFB_1. These properties are a high cation exchange capacity and large surface area. In fact, some clays in the Smectite family have been shown to have as much as 8 times more of these two properties than other types of clays. Two examples of Smectite clays are Bentonite and Montmorillonite which are very similar in their chemical and physical properties. In fact, they are so similar with regard to their properties that they are often considered to be the same clay. Thus, their names are often used interchangeably and synonymously. In some instances authors have promoted confusion in their articles written about the ability of different clays to bind AFB_1 because they used Smectite, Montmorillonite and Bentonite synonymously. All that needs to be remembered is when either of these names is used the authors are referring to a clay having similar properties which result in their ability to bind AFB_1.

A major characteristic of clays in the Smectite Family that contributes to their ability to bind AFB_1 is that Smectites are considered phyllosilicate clays because their structure is associated with defined layers often resembling plates, flakes or leaves stacked on top of one another. In fact, the term "phyllo" is Greek in origin, means "leaf", and is still used today to define a middle-eastern pastry consisting of tissue-thin layers of dough that becomes flaky when baked. Once stacked, spaces exist between the crystalline layers. If the clay's layers are wide enough apart molecules of different sizes and possessing an ideal conformation may easily enter the spaces. In the next issue of Milwhite's Journal the mechanisms of AFB_1 binding will be discussed along with other interesting information about clays.

Note: The information in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

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MILWHITE INC.
JOURNAL

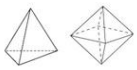
OCTOBER 2015

IMPROVED MILBOND-TX®

CLAY-BASED MYCOTOXIN BINDERS

"REASONS WHY IMTX IS AN EFFICIENT AFB_1 BINDER"

Aflatoxin B_1

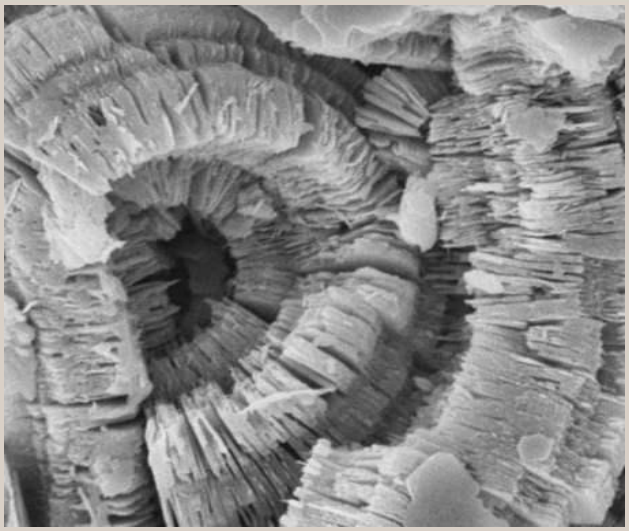


Tetrahedron/Octahedron

In the previous issue of Milwhite's Journal a simple to understand general overview of clays was presented with regards to their origin, nomenclature, formation and some of their chemical and physical properties which are responsible for certain clays being efficient AFB_1 enterosorbents (i.e., binders). Improved Milbond-TX® (IMTX) is a Smectite clay, classified as a "hydrated sodium calcium aluminosilicate" (HSCAS) with a physical crystalline structure consisting of layers resembling "plates or flakes" stacked on top of one another. It is this attribute along with the fact that Smectite clays are associated with a high cation exchange capacity (CEC) that makes IMTX such an efficient AFB_1 binder. Aluminosilicate clays are formed in various locations in the earth's crust from weathered volcanic ash rock and have a high concentration of alumina (Al_2O_3) and silica (SiO_2) along with the presence of basic cations such as Na+, K+, Ca++, Mg++ and Fe++. Smectite, is also referred to as "Fullers Earth Clay" or simply as "Fullers Earth" which, historically, has been used for thousands of years for cleaning. It was especially popular for cleaning and removing the waxy-oily component of wool called "lanolin". The process of cleaning the wool was called "fulling" and the individuals doing the cleaning were called "fullers". In fact, the term "Smectite" is Greek in origin and arises from the word "smēchein" which means "to clean". Other names which are commonly used by the animal feed industry to identify Smectite types of clays are "Bentonite" and "Montmorillonite" and because they have very similar chemical and physical properties, the names Smectite, Bentonite and Montmorillonite are often used interchangeably. Any of these names is acceptable to use when describing an aluminosilicate clay that has a high binding affinity for AFB_1. The qualities of IMTX that are responsible for its ability to sequester AFB_1 in the animal's digestive tract are discussed in this issue of Milwhite's Journal.

IMTX is an "expanding" or "swelling clay" because its structural properties allow water molecules to enter into the spaces between its many layers. Smectite clays, with regards to their capacity to bind AFB_1, are able to do so because of an ability to expand to many times their original volume, but all clays do not possess this property. The amount of interlayer expansion in Smectite clays is dependent on which cation is present. When the major interlayer cation is Na+ more expansion occurs because atomic bonding between the interlayers is not as strong allowing more water molecules to enter. When Ca++ is the major interlayer cation less expansion occurs due to stronger interlayer atomic bonding. For instance, Kaolinite is an aluminosilicate clay, just like IMTX, but is non-expandable. The reason is because Kaolinite is considered a 1:1 clay, whereas a Smectite clay, like IMTX, is a 2:1 clay. In order to understand what this means we must remember that the two basic components of clays are the elements Si and Al. As stated earlier, these two elements when combined with oxygen form molecules or "groups" with specific shapes.

For Si the shape is always a tetrahedron and for Al the shape is always an octahedron. These specific molecules (or "groups") link together by the thousands to form long tetrahedral sheets or long octahedral sheets. When talking about a "clay layer" a 1:1 clay layer always consists of one tetrahedral sheet bound to one octahedral sheet. (Note: An easy way to visualize this is to think of the tetrahedral sheet and the octahedral sheet as two sheets of paper on top of one another forming the layer). Accordingly, in a 2:1 clay each layer will always be formed by two tetrahedral sheets with one octahedral sheet sandwiched between them. Of course, the sheets in a 1:1 and a 2:1 clay must be held together to provide structural integrity to each layer. This is accomplished by the atomic bonding of elements "cations" associated with the sheet's negatively charged surface located between the layers as well as on the edges and outer surface of each sheet. Once stacked on top of one another the layers in a 1:1 clay (e.g., Kaolinite) do not expand because the atomic bonding (especially hydrogen bonding) holding the two layers together is very strong and does not allow water molecules to enter. This is why Kaolinite has a small amount of surface area and a low cation exchange capacity (CEC). Therefore, because of its properties Kaolinite is excellent for making ceramics, but is not an effective AFB_1 binder. In fact, the clay got its name from the Kao-Ling village in the Jiangxi province of China noted for high quality ceramic material known as "kaolin" which is the clay used to produce highly prized porcelain or "fine china". On the other hand, IMTX is an effective AFB_1 binder, but is not the clay of choice for use by the ceramics industry. In a 2:1 clay the atomic bonding (oxygen-oxygen and cation-oxygen) holding the layers together is weak and easily broken and allows water molecules to enter the spaces between the layers. It has been estimated that the average size of the unexpanded interlayer spaces in Smectites is between 1 to 2 nanometers, whereas in a 1:1 clay the size is only 0.7 nanometers.



Once expanded, the water-rich interlayer spaces of IMTX provide an enormous amount of surface area for binding and trapping AFB_1 molecules that enter these spaces. Also, the negative charges associated with the interlayer surfaces are balanced by the various cations within the interlayer spaces. Because of this, IMTX is known to possess a high CEC which is also responsible for attracting and binding AFB_1 molecules. Since a 1:1 clay does not have as much surface area and the binding of cations and AFB_1 is limited solely to the negative charges along the edges and outer area of each layer. It has been estimated that the effective surface area for one gram of 1:1 clay is only 10-30 square meters, whereas in a 2:1 clay like IMTX, the effective surface area is from 650-800 square meters/gm of clay.

Smectite clays, when expanded, provide sufficient space for AFB_1 molecules to enter the interlayer spaces. A molecule of AFB_1 has a flat-planar configuration with an estimated size of 12.8 X 10.4 angstroms which makes it smaller than many other mycotoxins. Clays containing a crystalline structure with interlayer spaces smaller than these dimensions are, therefore, limited in their effectiveness as binders of AFB_1 since effective binding in these clays is limited only to the clay's external surface and not the interlayers. The bonding between divalent cations and AFB_1 occurs at the carbonyl (C=O) groups of AFB_1 molecules (see figure). The high CEC and large surface area associated with Smectite clays are the two main driving forces behind their ability to attract and bind AFB_1 and other toxins and contaminants.

So, putting all of this in perspective, when a sufficient concentration of IMTX is present in the liquid medium within an animal's digestive tract, in the presence of AFB_1, the chemical and physical properties associated with the crystalline structure of IMTX promote expansion of its interlayers providing spaces for AFB_1 molecules to enter more easily. Once within these water-rich spaces formed by these interlayers adsorption of AFB_1 occurs with specific atoms associated with its crystalline structure just as adsorption of AFB_1 occurs on the outer surfaces and edges of IMTX. Once adsorbed to the outer surfaces and trapped between the interlayers of IMTX, the AFB_1 molecules will eventually be excreted in an animal's feces, thus resulting in no decrease in animal performance which is known to be associated with aflatoxicosis

Note: The information in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

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MILWHITE INC.
JOURNAL

NOVEMBER 2015

IMPROVED MILBOND-TX®
MYCOTOXIN BINDERS

"ALL CLAY-BASED ADDITIVES ARE NOT THE SAME"

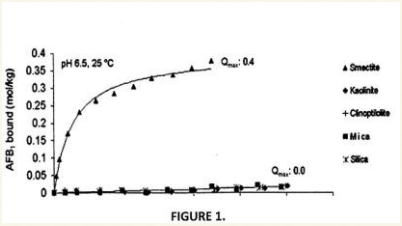


FIGURE 1.

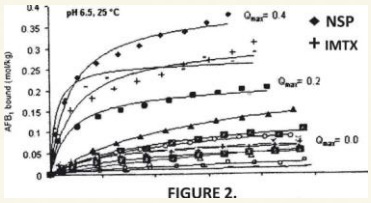
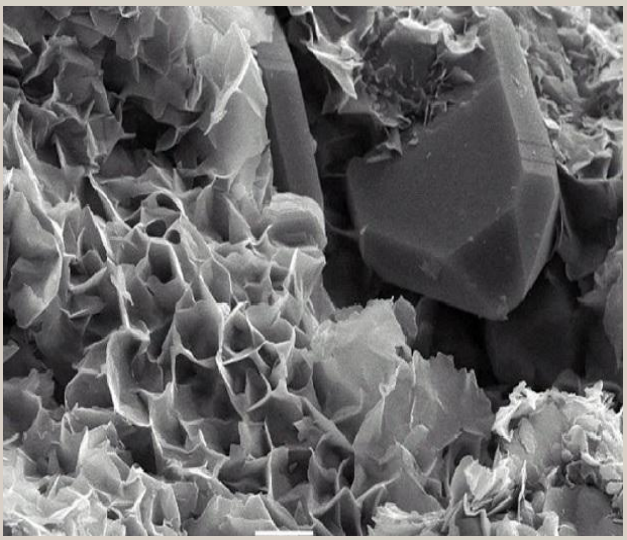


FIGURE 2.

If a specific clay is believed to possess the necessary properties to be considered a good candidate for adsorbing (binding) one or more mycotoxins the normal procedure is to eventually subject the clay to *in-vitro* mycotoxin binding tests. If these *in-vitro* screening tests prove that a specific clay has a high degree of mycotoxin binding capability it does not necessarily mean that the clay is going to be an effective binder of one or more mycotoxins *in-vivo*. Follow up studies are required with the more essential and costly *in-vivo* tests using an animal species that will be consuming the clay once it is added to an animal's diet. Improved Milbond-TX® (IMTX) has been extensively tested since 1999 for its ability to successfully bind aflatoxin B_1 (AFB_1). In previous issues of Milwhite's Journal all of the *in-vivo* safety and efficacy studies with IMTX, conducted in different university trials using different animal species, have been the topics of discussion. Many of the additives in the commercial marketplace that companies claim to be mycotoxin binders are periodically tested in comparison studies to determine, if indeed, their claims are true and they have the necessary properties to bind certain mycotoxins and specifically AFB_1. Such a comparison study was conducted by Marroquin-Cardona and colleagues in 2009. These investigators conducted *in-vitro* tests that characterized and compared 12 different additives all of which were being sold in Mexico as mycotoxin binders and claiming to have the ability to bind AFB_1. A few of these additives had claims of being able to bind multiple mycotoxins. Names of the additives and the experimental results obtained for each additive, as well as a more detailed description of the tests to which all 12 additives were subjected in this comparison study can easily be found in the cited publication of Marroquin-Cardona et.al. (2009) and Figures 1 and 2 presented here were adapted from their publication.

Using X-ray diffraction analysis to characterize the crystalline structure of these clays, 9 were confirmed to contain Smectite, but the effectiveness of the AFB_1 binding ability among the 9 clays was considerably different. This means that even though clays contain Smectite, it does not necessarily mean that such clays will have a similar ability to bind AFB_1 because there are other factors that are contributing to their AFB_1 binding ability. Or, another way to think about this is that other factors are exerting a negative influence on the ability of a Smectite clay to efficiently bind AFB_1. From the data collected in their study, these authors concluded that two of the major factors contributing to a high AFB_1 binding potential were the particle size in the clay additive and the presence of the clay mineral, montmorillonite, in Smectite. The properties of montmorillonite that contribute to the high AFB_1 binding potential of Smectite clays was discussed in a previous issue of Milwhite's Journal.

Since kaolinite, mica, silica, and clinoptilolite may have been present in the 12 clay-based additives tested, these investigators decided to determine their ability to bind AFB_1. Reference standards of these 4 minerals were obtained from the Clay Mineral Repository at Purdue University in West Lafayette, Indiana, USA. In order to test their capacity to adsorb AFB_1, 11 initial solutions were developed containing increasing concentrations of AFB_1 ranging from 0.4 to 8.0 ppm in 5 ml and 0.1 mg of each mineral was added to each solution. The pH and temperature of each solution was 6.5 and 25°C, respectively. Suspensions of these solutions were formed and following dilution, incubation at 25°C for 2 hours and final centrifugation, the amount of AFB_1 remaining in solution was determined. Qmax was determined for each additive. Qmax is simply a term that is used to express the AFB_1 binding ability of a feed additive. Qmax is expressed as mol/kg (i.e., moles of AFB_1 bound/kg of additive) and can be found on additive labels in the commercial marketplace. Qmax is considered an important required criteria when companies apply for additive licensing. During the past 30 years, research data from numerous studies have shown that the above 4 minerals have a minimal ability to adsorb AFB_1. Confirmation of the inability of these minerals to bind AFB_1 is shown in Figure 1. Only Smectite was shown to have an ability to adsorb AFB_1. This should come as no surprise since Smectite clays that possess the highest AFB_1 adsorptive capacity are also associated with having high concentrations of montmorillonite.



Therefore, a major characteristic of the feed additives tested in this study and having the highest ability to adsorb AFB_1 was to contain a high concentration of montmorillonite and IMTX contains approximately 85% montmorillonite.

Of the 9 clays that contained Smectite only 4 exhibited acceptable abilities to bind AFB_1. In Figure 2, the capacity of each clay to bind AFB_1 is presented. The procedure followed for these 12 samples to determine their adsorptive capability of AFB_1 was the same as that used for the 4 minerals discussed above. It can be noted that of the 12 clays, IMTX had the second highest Qmax which was greater than 0.3 mol AFB_1/kg.

From this comparison study of 12 additives, the authors concluded that the following attributes contributed to a clay's ability to effectively bind AFB_1. It should be pointed out here that IMTX has all of these attributes. Proper particle size has a major influence on the AFB_1 binding effectiveness of an additive. The active ingredient in a clay with a high AFB_1 binding capability is Smectite (i.e., montmorillonite). The effectiveness of AFB_1 binding in an additive is affected if contamination of Smectite occurs resulting in dilution as a result of sand, silt, organic matter and minerals that have a negative effect on the binding ability of a clay. The capability of a clay to adequately expand or swell in the presence of water is an essential characteristic and montmorillonite is considered an expandable clay. With adequate "swelling volume" (SV), molecules of AFB_1 can easily enter the interlayer spaces of Smectite clays noted for their highly layered structure. In fact, in this comparison study, the additives that had the lowest SV or no SV at all had the lowest ability to bind AFB_1 (i.e., lowest Qmax).

One of the most extensive studies to ever be conducted and reported on the ability of Smectite clays to adsorb AFB_1 is the M.S. Thesis of Kannewischer (2006). In this research, 20 smectite clay samples were tested for their chemical and physical properties and their properties related to the effectiveness to adsorb AFB_1. Discovered was the fact that differences in Smectite composition are responsible for differences in their adsorption capabilities and not so much the relative abundance of a Smectite clay. Among the Smectite samples tested there existed as much as a 10-fold ability to adsorb AFB_1. A major conclusion from this extensive research study involving 20 Smectites was that the greatest ability to adsorb AFB_1 resulted when a Smectite clay was mostly composed of montmorillonite.

Note: A complete description of the experiments mentioned in this issue and all of the data collected in the experiments conducted by the authors can be found in the referenced publication located in the footnote below. The information compiled in this issue of ***Milwhite's Journal*** *was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.*

Marroquin-Cardona, A., Y. Deng, J.F. Taylor, C.T. Hallmark, N.M. Johnson and T.D. Phillips. 2009. In-vitro and in-vivo characterization of mycotoxin-binding additives used for animal feeds in Mexico. Food Additives and Contaminants, Vol. 26, No. 5, May 733-743.

Kannewischer, Ines. 2006. Smectite clay adsorbents of aflatoxin B_1 to amend animal feed. Master's Thesis, Department of Soil Science, Texas A&M University.

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MILWHITE INC.
JOURNAL

DECEMBER 2015

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"VANADIUM: KEEP IT OUT OF LAYING HEN DIETS"

FACTORS AFFECTING EGG QUALITY

This issue of **Milwhite's Journal** is the first in a series of four dedicated to discussing the influence of vanadium (V) on overall egg quality and egg shell pigmentation. The importance of keeping the concentration of V as low as possible in commercial egg-type laying hen diets often needs to be reviewed because of the negative effects that V has been shown to have on egg quality. If not reviewed from time-to-time and egg quality issues arise within a poultry company, V may not be remembered or even recognized as a possible cause since there are numerous other factors that are known to cause egg quality problems and these are often blamed without considering all possibilities (i.e., V).

Age of the bird, mineral/vitamin nutrition, disease/parasites and length of egg storage and storage temperature will influence egg quality. Two major stressors that are known to negatively affect egg quality are high environmental temperature and mycotoxins in the feed. Elevated temperature will affect both normal shell formation and internal quality characteristics of eggs. Mycotoxins such as the nephrotoxin, Ochratoxin A, and the hepatotoxin, Aflatoxin B_1, as well as T-2 toxin, Fumonisin, Zearalenone and Citrinin have all been shown in research studies and in the field to negatively affect egg quality. Decreased quality characteristics that are commonly associated with mycotoxins are thin and abnormally shaped egg shells, decreased albumen height and decreased yolk weight and pigmentation.

Egg quality means different things to different people since consumer acceptability and preference standards have a major influence on purchasing decisions. There are two main attributes of eggs which are associated with their quality and these are their external and internal characteristics. The shell is the external characteristic evaluated for quality and the albumen and yolk are the internal characteristics evaluated for quality. Vanadium is known to cause problems with the normal development of the characteristics associated with these two attributes. It is also important to remember that V has not only been shown to have a negative effect on the quality of egg albumen, but it is now known that V is also detrimental to the development of normal shell pigmentation of eggs from hens laying brown shelled eggs.

VANADIUM SOURCE

High quality feed-grade sources of phosphate are always expected to contain very low concentrations of V. However, when world-wide feed-grade phosphate prices increase, the cheaper inferior sources of feed-grade phosphate often find their way into the feed ingredient market. High-V phosphate sources are known to originate from any part of the world where raw rock-phosphate deposits are mined. Usually, geologic phosphate deposits have a higher V concentration than marine phosphate deposits. Concentrations as high as 6,000 mg V/kg have been reported in some rock phosphate deposits in various parts of the world. It is doubtful that a phosphate source with such a high V concentration would ever be intentionally used as a phosphorus source in any poultry diet and removal of these high V concentrations are essential before such sources could be used in any animal diet. Most marine phosphates contain approximately 120 mg/kg V whereas, geologic deposits could possibly contain from 1,000 to 2,000 ppm V or more. Good quality sources of mono-calcium phosphate, di-calcium phosphate, tri-calcium phosphate, etc. generally contribute less than 2 mg/kg V to the diet of laying hens and diets of other types of poultry.

Normally, laying hen diets contain concentrations of V that would be of no concern to nutritionists and egg producers. There are times however, when the diets of laying hens and other poultry may contain a poor quality source of phosphate that contributes a high concentration of V to the diet. In these cases, problems with albumen quality and egg shell pigmentation can be directly attributed to V. Vanadium is a "transition" element that has four valence states (+5, +4, +3 and +2) and for that reason V has strong oxidation potential and therefore promotes oxidative stress within animal cells.

Vanadium has been shown to act as a catalyst in the oxidation of certain organic compounds such as membrane phospholipids and has also been shown to inhibit the enzymatic actions of pepsin and trypsin during protein digestion in the stomach and lumen of the intestine, respectively. It is because of the high oxidation potential of V that its detrimental mechanism of action in animals is partly through inhibition of cellular enzymes and cell damage from lysis. In fact, it has been speculated that the deterioration of interior egg quality characterized by a low albumen height caused by V is mediated by muscle atrophy leading to an inhibition of motility in the magnum, which is the albumen-secreting portion of the hen's oviduct.

Miles and Henry (2004) reviewed the effect of V on egg interior quality and reported data from their laboratory documenting the effect of time and storage conditions on interior quality of eggs from hens fed diets containing 0 or 10 mg V/kg. Hens fed V had poorer albumen quality than hens not fed V and storing collected eggs in the layer house at ambient temperature rather than in a cooler (15.5°C, 60% relative humidity) decreased albumen quality at a faster rate. However, these researchers reported that V had no effect on the rate of decline in either environment. Implications of this finding are of importance in countries where government regulations do not require eggs to be refrigerated. Even though consumption of V would result in poorer albumen quality the deterioration of albumen quality would not be expected to increase at a faster rate if the eggs laid by hens consuming V were stored at ambient temperature.

The maximum tolerable concentration of V for poultry is listed in many literature references as 10 mg V/kg diet. However, research data have shown that problems with albumen quality will more than likely begin to occur when the concentration of V is 5 mg V/kg diet or higher. If a decision is ever made to use high-V phosphates during a crisis situation then they should cautiously be used in diets for ruminants rather than poultry because V is better tolerated by ruminant animals. Ruminants have a maximum tolerance in the diet of 50 mg V/kg. In the next issue of **Milwhite's Journal** the topic of discussion will center on the negative influence that V has been found to have on the pigmentation of brown shelled eggs.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Miles, R.D. and P.R. Henry. 2004. Effect of time and storage conditions on interior quality of eggs from hens fed vanadium. J. Appl. Poultry Research. 13: 619-627.

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MILWHITE INC.
JOURNAL

JANUARY 2016

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"VANADIUM: A FACTOR RESPONSIBLE FOR DEPIGMENTATION OF BROWN SHELLED EGGS"

EXPECTED EFFECTS OF VANADIUM IN LAYER DIETS

The adverse effects of vanadium (V) in laying hen diets have been reported by numerous investigators in several scientific journals over the past 50 years. Dietary concentrations of V used in previous experiments have ranged from 0 to 250 mg V/kg, with the majority of the investigators reporting the effects of 100 mg V/kg or less. Feeding laying hens a diet containing 30 mg V/kg or higher usually results in a decrease in feed intake, egg production, body weight, egg weight, fertility, hatchability and also poor feed conversion and increased excreta moisture. Bone, kidney, liver, and oviduct (magnum) are primary sites of V accumulation in laying hens.

Previously, the most common detrimental effect of dietary V in layers has been documented to be the decrease in interior egg quality as a result of low albumen height. A decline in albumen quality usually occurs within 48 hours after hens consume a diet containing 5 mg V/kg or greater and the decline in quality is thought to be mediated by muscle atrophy leading to an inhibition of motility in the magnum, which is the albumen-secreting portion of the hen's oviduct. Numerous researchers have reported that supplementing a diet containing 20 mg V/kg or less with 100 mg vitamin C/kg will, in most cases, overcome the negative effect that V has on albumen quality and completely restore albumen height. The time required to restore albumen height after removing V from a hen's diet is related to the concentration of V and the length of time the diet has been fed to the hens. When diets containing less than 15 mg V/kg are fed to laying hens, restoration of albumen quality usually occurs within 3 or 4 days. However, when higher than 30 mg V/kg diet are fed it may take up to 30 days for egg interior quality to be completely restored.

THE DEPIGMENTING EFFECT OF VANADIUM ON BROWN SHELLED EGGS

There are several factors known to cause a decline in the pigmentation of brown egg shells. Stress level of the flock, age of the laying hen, disease (especially infectious bronchitis), and chemotherapeutic agents such as sulfonamides have been reported to decrease brown egg shell pigmentation. The first report in the scientific literature documenting the depigmenting or "bleaching" effect of brown eggshells caused by V was from the laboratory of Dr. Richard Miles at the University of Florida Gainesville, Florida (USA) (Sutley et.al. 2001).

In their first study, these researchers used replicate floor pens of broiler breeders and supplemented their diet with 0, 50 or 100 mg V/kg as the compound "ammonium meta-vanadate" for a period of only seven days. A computer assisted color vision system (above photograph) was used to determine pigmentation in each egg. Within five days of feeding V there was a statistically significant ($P < 0.05$) decrease in the amount of brown pigmentation in the egg shells. Having previously established the negative effect on egg shell color using broiler breeders, the researchers then designed two similar experiments using Hy-Line Brown commercial egg-type layers (Odabasi et al. 2006). These hens were fed a corn-soybean meal basal diet adequate in all nutrients and energy and again supplemented with 0, 50, or 100 mg V/kg as the compound "ammonium meta-vanadate" in order to determine if V would also have a negative effect on egg shell pigmentation similar to that observed in the broiler breeders. In their second study using Hy-Line Brown hens, the researchers used a similar basal diet containing lower dietary levels of V (0, 15 or 30 mg V/kg).

In both of the above experiments with commercial egg-type brown layers there was a significant negative "bleaching" effect ($P < 0.05$) observed in egg shell pigmentation within three days of feeding hens the diets containing 30 mg V/kg or higher. Eggs from hens fed 15 mg V/kg diet also showed a bleaching effect, but not as great as for the higher V concentrations.

Even though eggshell pigmentation of the broiler breeder egg is not of any economic importance to the poultry industry, this is not true for the brown table-egg industry where shell color intensity and uniformity of color among eggs influence consumer preference. For example, in the Japanese egg market there are rigid standards for a uniform dark shell color, whereas other markets around the world prize a uniform light brown tint to the egg shell. Uniformity of shell pigmentation and color intensity among brown eggs are very important. Whenever pigmentation problems occur in flocks of brown egg-type hens another factor has been added to the list that might be responsible for the loss in pigmentation and that factor is V. Most of the feed ingredients used in poultry feed formulation contain very low concentrations of V. It is therefore, unlikely that V will be the factor responsible for causing a problem of pigmentation of brown-shelled eggs unless the diet contains a feed-phosphate source that has a high concentration of V.

The data collected in research studies at the University of Florida have shown that V is another factor which should be added to the list of factors that are responsible for depigmentation of brown-shelled eggs. In the next issue of Milwhite's Journal the topic will focus on how egg shell pigmentation can be completely restored even though V is in the diet of brown egg-type layers.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Sutley, J.P., R.D. Miles, C.W. Comer and M.O. Balaban. 2001. The influence of vanadium on pigmentation of brown-shelled eggs. Poultry Sci. 80:1039
Odabasi, A.Z., R.D. Miles, M.O. Balaban, K.M., Portier, and V. Sampath. 2006. Vitamin C overcomes the detrimental effect of vanadium on brown eggshell pigmentation. J. Appl. Poult. Res. 15:425-432.

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MILWHITE INC.
JOURNAL

FEBRUARY 2016

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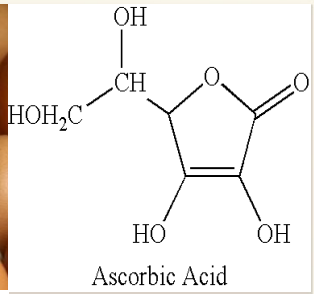
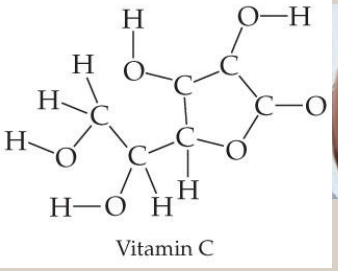
"OVERCOMING THE DETRIMENTAL EFFECT THAT VANADIUM HAS ON BROWN EGG SHELL PIGMENTATION"



Ascorbic Acid



Vitamin C

In the previous issue of Milwhite's Journal the topic of discussion highlighted the research conducted at the University of Florida, Gainesville, Florida (USA) with broiler breeders and Hy-Line Brown commercial egg-type layers documenting the depigmenting ability that vanadium (V) has on brown shelled eggs. These researchers fed V concentrations of 0, 15 and 30 mg V/kg diet in a corn soybean meal diet to Hy-Line Brown layers. These dietary concentrations of V were used because the researchers felt that even if diets were supplemented with a poor quality feed-grade phosphate source, the phosphate source would normally not contribute more than 30 ppm V to the diet. On days 3, 6 and 9 of a nine day period in which the three experimental diets were fed, each of the shells of the eggs collected from Hy-Line Brown egg-type laying hens was analyzed for their pigmentation by a computer color vision system. This established the base-line egg shell pigmentation for eggs laid by hens fed each dietary treatment.

A significant decrease in egg shell pigmentation was observed to occur by day 3 and continued until day 9 when the diets were changed to diets containing three different antioxidants to determine if the antioxidants would restore the brown color to the egg shells. Assisted by the computer color vision analysis system the researchers were able to determine the actual cause of the depigmentation "bleaching" effect which occurred in the egg shells. Vanadium caused a reduction in the "red" component of the egg shell rather than the "yellow" component. Also, these researchers were able to determine the influence that V had on eggs with different "shades" of shell coloration (i.e., dark brown vs. light brown). When V was added to the diets of hens laying eggs with dark or light brown shells, the magnitude of decline in pigmentation was essentially the same. On day 9, the diets were changed so that the hens receiving the V supplemented diets received a diet also supplemented with one of the following antioxidants: none, 100 mg vitamin C/kg, 100 IU vitamin E/kg, or 100 mg beta-carotene/kg. Eggs collected on day 3, 6, 9, 12, and 15 following the diet change were used for shell color analyses.

Results of the above experiment indicated that when vitamin C, vitamin E, and beta-carotene, were supplemented to the diets containing either 15 or 30 mg V/kg, only vitamin C totally restored egg shell pigmentation. Vitamin E and beta-carotene had no influence on restoring pigmentation to the egg shells. Further analysis of the data indicated that vitamin C restored the "red" component of the egg shell. Supplementing the antioxidants alone or in combination to the corn/soybean meal control diet containing no V had no effect on egg shell pigmentation.

Whether V is directly involved in enzyme or cofactor inhibition within the tubular gland cells of the uterus, which results in deposition of less pigment in the shell, or it modifies the pigment molecules structurally at other sites before being delivered to the shell gland for deposition is not known. Also, the exact mechanism responsible for the complete restoration of egg shell pigmentation by vitamin C is not known. However, unlike vitamin E and beta-carotene being lipid soluble, vitamin C was the only supplemental antioxidant that was water soluble. This may have been a contributing factor because, being water soluble, vitamin C was more available to directly prevent or assist in preventing V induced oxidative damage associated with enzyme-catalyzed pigmentation reactions occurring in the cytoplasm of the uterine (shell gland) cells.

If a crisis situation occurs and the use of a poor quality high-V phosphate source is unavoidable, egg producers should expect to see a deterioration of egg albumen quality in eggs from hens laying white or brown shelled eggs and also decreased shell pigmentation in brown eggs. However, we now know that research data in the scientific literature clearly document that supplementing 100 mg vitamin C/kg diet will restore egg albumen quality in situations where hens are consuming diets containing 30 mg V/kg or less. Also, in a field situation, the "bleaching" effect of brown egg shells which occurs when brown egg-type laying hens consume a diet containing 30 mg V/kg or less can be overcome. A complete restoration of normal egg shell pigmentation would be expected when vitamin C is supplemented to the diet at 100 mg vitamin C/kg. Data collected in other experiments by Florida researchers have shown that supplementing 100 mg vitamin C/kg diet "before" or "at the same time" or "after" V is introduced to the diet will prevent a decline in egg shell pigmentation. The next issue of Milwhite's Journal focuses on shell pigmentation and explains why pigmentation decreases as hens age.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Odabasi, A.Z., R.D. Miles, M.O. Balaban, K.M., Portier, and V. Sampath. 2006. Vitamin C overcomes the detrimental effect of vanadium on brown eggshell pigmentation. J. Appl. Poult. Res. 15:425-432.

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JOURNAL

MARCH 2016

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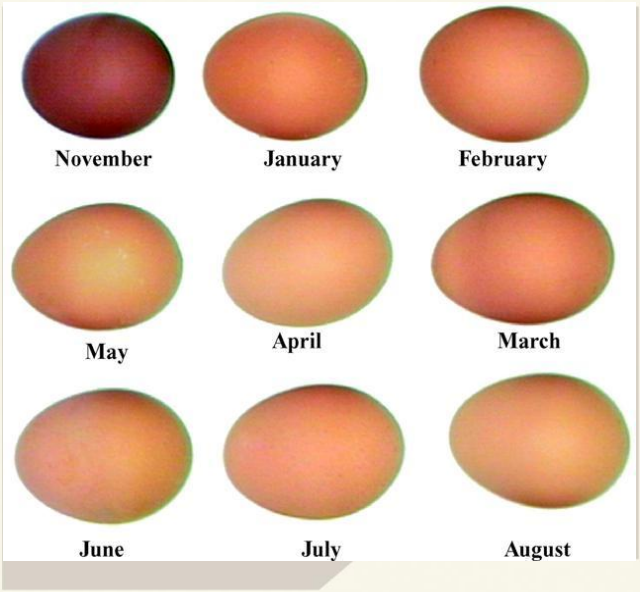
"WHY BROWN EGG SHELL PIGMENTATION DECREASES WITH AGE OF A LAYER FLOCK"

Although shell pigmentation (color) is not an indication of internal egg quality, consumers in many egg markets throughout the world prefer brown over white eggs (e.g., United Kingdom, Italy, Portugal, Ireland, Southeast Asia, Australia and New Zealand). Shell color intensity within each country is highly influenced by consumer preference. For example, the Japan egg market has standards for a uniform dark shell color, whereas consumers in other markets prefer a shell with a uniform light brown color. One would think that because egg shell color is of extreme importance in many egg markets there would have been extensive research studies aimed at explaining reasons for the variation in shell color that occurs among and within layer flocks, especially changes that occur as the laying cycle continues. In spite of the economic losses resulting from variations in shell color, the reasons for changes in color as laying hens age were not completely understood until research conducted at the University of Florida, Gainesville, Florida (USA) helped explain why color loss occurs as laying hens age (Odabasi et al. 2007). The reason why egg shell color declines as laying hens age is similar to why egg shell thickness declines as hens age. Even though the reason why egg shell thickness decreases with the age of the hen will be a topic discussed in a future issue of Milwhite's Journal it is appropriate to review the reason why at this time to help explain why egg shell color declines as well.

Dr. David Roland, while a faculty member in the Department of Poultry Science at the University of Florida, Gainesville, Florida (USA) and his coworkers, published research data that explained the reason why egg shell quality declined with the age of the laying hen (Roland, et.al., 1975). These researchers reported that as the hen aged, the normal increase in egg size was accompanied by no proportionate increase in the total amount of calcium deposited on the egg shell. Simply stated, approximately the same amount of calcium carbonate that covered a small egg from a young pullet covered a large egg from an older hen. Then, in 1979 Dr. Roland published another paper (Roland, 1979) that greatly expanded the knowledge in the area of egg shell quality. He reported that eggs which had a greater increase in size throughout the entire laying cycle also had a greater decline in their shell quality. Further research led to the discovery that in a layer flock the quality of a hen's egg shell at the end of the laying cycle was directly related to the shell quality of her eggs at the beginning of the cycle. Also, the number of eggs a hen laid during the production cycle had no influence on the quality of the egg shells.

EGG SIZE AND SHELL COLOR

Just as Dr. David Roland concluded in 1975 that the decline in egg shell quality with age of the hen is a direct result of an increase in egg size without a proportionate increase in calcium carbonate deposition in the egg shell, the research data collected and reported by Odabasi et.al., 2007) showed that the decrease in egg shell color as a brown hen ages is also directly attributed to an increase in egg size without an accompanying increase in the amount of shell pigment deposition. These researchers used brown egg-type layers and measured the change in brown color in egg shells for 10 months using a computer-based color machine vision system.
The actual color and color intensity was determined for three eggs collected each month from each of 240 hens. Data collected during the 10-month experimental period showed that as the hen aged her eggs became lighter in color and the decrease in color was due to less intensity of a red pigment in the shell (above photograph). Their research data also showed that hens laying eggs with less pigmentation during the early part of the laying cycle laid lighter colored eggs at the end of the laying cycle.



These data, relating eggshell color changes with hen age, are in total agreement with those reported by Dr. Roland about egg shell quality. Hens laying eggs with poor shell quality in the early part of the laying cycle also laid eggs with poor shell quality later in the laying cycle. Similarly, in this present study concerning egg shell pigmentation, hens that laid eggs with more pigment on the shell (darker shells) early in their laying cycle continued to lay darker eggs with more pigment towards the end of the laying cycle.

At the end of their 10-month study the Florida researchers corrected the egg pigmentation data for the increase in egg weight and found very little change in egg shell pigmentation occurred between the first and the last month of the 10-month experimental period. Therefore, the larger egg shell surface area due to the increase in egg size resulted in lighter colored eggs and the decline in shell pigmentation is normal and should be expected in brown layers as their laying cycle continues. However, it must be kept in mind that there are other factors that have a negative effect on egg shell color as discussed in a previous issue of Milwhite's Journal. These factors must be understood and controlled so that the impact they have on the normal decline in egg shell pigmentation as a flock of brown egg-layers get older will be minimal.

It is normal and should be expected that as a flock of white and brown egg-type layers age their eggs get larger and their egg shells become thinner. This leads to a decline in egg shell quality with age of the flock. Similarly, as a flock of brown egg-type layers age and their egg size increases the intensity of the brown color in the egg shell decreases. The explanation for this is because the same amount of pigment, as deposited on a small egg, is being deposited onto a larger egg that has more surface area. So, this means that once corrected for egg weight, there is very little change in the total amount of pigment in the egg shell as the hens get older and the laying cycle continues.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

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APRIL 2016

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UNDERSTANDING PHOSPHATE SOURCES

"REMOVING A MAJOR MISCONCEPTION ABOUT FEED-GRADE PHOSPHATES"

OVERVIEW

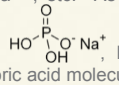
The animal feed industry throughout the world uses millions of tons of high quality commercial sources of inorganic feed-grade phosphate each year. The relative bioavailability of the phosphorus (P) as well as the calcium (Ca) in these high-quality products is usually high. Even though the guaranteed amount (%) of P and Ca in these commercial phosphate sources is known, the purchaser is normally not aware of the exact chemical form in which the P exists. However, using X-ray diffraction techniques and other quality control methods, companies manufacturing feed-grade phosphates are able to determine the actual form and quantity of P and other minerals present in their products. In this issue of Milwhite's Journal a major misconception about Ca phosphate sources is clarified and especially the mistaken belief about dicalcium phosphate that exists among most people throughout the world. Elemental structures are provided for a visual representation of the molecules even though the natural atomic bonding within the molecular structures is different than those presented here for simplification purposes.

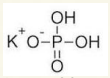
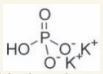
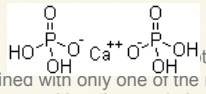
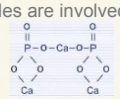
Three major sources of inorganic P are used in the worldwide animal feed industry.These three sources are commonly referred to as monocalcium phosphate, dicalcium phosphate and tricalcium phosphate. It is very common for nutritionists and feed manufacturers to also refer to these three sources of P as MONOCAL, DICAL and TRICAL, respectively. However, almost no one within the animal feed industry as well as in academia seems to remember that from a chemical standpoint there is a more correct way to refer to these three phosphate sources. These "chemically correct" names are monobasic calcium phosphate, dibasic calcium phosphate and tribasic calcium phosphate. It is because the word "basic" has been forgotten and eliminated when referring to these sources that a misconception exists with regards to these high quality phosphate sources.

THE MISCONCEPTION: Most everyone assumes when referring to mono, di and tricalcium phosphate that the prefixes "mono", "di" and "tri" are indicating the number of Ca atoms in the phosphate source. So, using this logic, it is easy to understand why people that have been asked the question: "How many calcium atoms are in MONOCAL, DICAL and TRICAL? ", answer by saying, "they contain one, two and three atoms of Ca, respectively." However, this is not true. DICAL (dicalcium phosphate) only contains one Ca atom even though MONOCAL and TRICAL, do indeed, contain one and three Ca atoms, respectively. In fact, "mono", "di" and "tri" are not referring to the number of Ca atoms nor to their relative bioavailability, but instead are referring to the particular properties of the phosphoric acid molecule.

At this point, in order to clarify the misconception, it is essential to understand to what the word "basic" is referring with regards to the properties of the phosphoric acid molecule. First, we must consider a molecule of phosphoric acid which has the molecular formula, H_3PO_4  , and remember in chemistry we learned that a molecule that donates hydrogen to a solution is referred to as an "acid" and the donated hydrogen is referred to as a "proton" which is simply a hydrogen atom without an orbital electron. On the other hand, a "base" accepts protons. So, chemically speaking, the term "basic" is used here to denote how many hydrogens have been displaced (removed) from a molecule of phosphoric acid. For instance, if one hydrogen is removed the phosphoric acid molecule is referred to as "monobasic phosphate" with the formula of H_2PO_4- or 

If two hydrogen's are removed the molecule is referred to as "dibasic phosphate" with the formula HPO_4= or  nd when all three hydrogens are removed the phosphate is in the "tribasic" form (i.e.,"tribasic phosphate", or  .

Being in the mono, di or tri basic form now allows for the formation of a specific mineral salt. Salts of P can easily be formed by reacting with monovalent cations such as Na+ and K+ and divalent cations such as Mg++, Zn++, Ca++, etc. As an example, if sodium phosphate is formed the formula would be NaH_2PO_4,  , keeping in mind that only one hydrogen has been displaced from the phosphoric acid molecule, which gives rise to the more appropriate chemical name of "monobasic sodium phosphate".

Another example, using K+ to form mono, di, and tribasic potassium phosphate, respectfully, is as follows:    . So, basic atoms or groups have the ability to combine with the phosphate molecule depending on how many of its hydrogens have been displaced.When Ca++ reacts with phosphoric acid to form MONOCAL, DICAL and TRICAL the formula and structure of each is as follows: Monobasic calcium phosphate (MONOCAL), $Ca(H_2PO_4)_2$  tice that only one Ca atom is involved and that it has combined with only one of the negative charges of each phosphate in order to satisfy the two positive charges in its valence. Often, monobasic calcium phosphate is also referred to as "calcium biphosphate". However, when dibasic calcium phosphate (DICAL), $CaHPO_4$) is formed the single Ca atom attaches to two negative charges of only one phosphate as seen here and this is why it is sometimes referred to as "calcium monohydrogen phosphate"  . In order to form tribasic calcium phosphate (TRICAL), $Ca_3(PO_4)_2$, three Ca atoms and two phosphate molecules are involved in order to balance out their positive and negative valences, 

SUMMARY

Through the years, the word "basic" has been eliminated when referring to mono, di and tricalcium phosphate and this has led to erroneously interpreting the true chemistry of these phosphate sources, especially dicalcium phosphate. Even though in chemistry "di" indicates two of something, it does not mean that there are two atoms of Ca in a molecule of DICAL. In this case the "di" is simply referring to the basic nature of the phosphate molecule because it has lost two of its hydrogens and in-so-doing is capable of forming mineral salts with monovalent and divalent cations. Hopefully, the above information has assisted in clarifying the misconception about these phosphate sources and in the future when you hear someone ask the question, "How many Ca atoms are in dicalcium phosphate?" it will be easier to understand why most people answer the question by saying, "two", which you now know is the wrong answer.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

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UNDERSTANDING FEED-GRADE CALCIUM PHOSPHATE SOURCES
"A BRIEF GENERAL OVERVIEW ABOUT THEIR PRODUCTION"

INTRODUCTION

In the last issue of Milwhite's Journal the topic selected for discussion addressed and clarified a major misconception that exists about the feed-grade phosphates, mono- di- and tricalcium phosphate. The reason this topic was selected was because even though each year animal feed-grade phosphates account for only about 5% of the world's total phosphate consumption, monocalcium phosphate (MCP) and dicalcium phosphate (DCP) accounted for over 90% of the world's feed-grade phosphate usage. Also, there seems to be a void of meaningful discussions regarding the basic chemistry of these phosphate sources as well as the manufacturing processes involved in their production. During the past few decades, meaningful discussions on this topic have been very limited in college and university classrooms as well as in the many nutrition conferences and symposia sponsored each year within the animal feed industry. Therefore, along with the information presented in the previous issue of Milwhite's Journal, this issue presents its readers with a brief general overview of the manufacturing process involved in production of these high quality feed-grade phosphorus sources in order to promote a better appreciation for their use by the world-wide animal feed industry.

MONOCALCIUM AND DICALCIUM PHOSPHATE

As pointed out and clearly explained in the previous issue of Milwhite's Journal, the prefixes "mono" and "di" in front of calcium phosphate are often erroneously interpreted to mean one and two atoms of calcium in the products commonly referred to in the animal feed industry as MONOCAL (MCP) and DICAL (DCP). The reader should refer to the previous issue of this journal for a complete explanation and understanding of the definition of MCP and DCP. In reactions I and II below, the mineral involved in the production of the phosphate salt is calcium from calcium carbonate (limestone) and depending on the final product will displace either one or two hydrogens from phosphoric acid. When dibasic calcium phosphate is formed, in reaction I, two hydrogens are displaced from a molecule of H_3PO_4 and the phosphate species has a valence of "— 2" (i.e., $HPO_4^{=}$). In reaction II only one hydrogen is displaced from each of two phosphoric acid molecules in the formation of monobasic calcium phosphate and the phosphate species has a valence of "— 1" (i.e., $H_2PO_4^{-}$).

During the manufacture of feed-grade calcium phosphates, the limestone ($CaCO_3$) and phosphoric acid (H_3PO_4) react together under carefully controlled industrial conditions. When these two ingredients are mixed together, a chemical equilibrium is reached and the final product is a mixture containing monobasic calcium phosphate (MCP) and dibasic calcium phosphate (DCP). When the reaction is complete the final mixture in the reaction vessel is removed, dried, ground and screened in order to assure a specific particle size for the feed industry.

It is sometimes confusing when the phosphoric acid used in the production of feed-grade phosphate sources is often referred to by manufacturers as "orthophosphoric acid". When this is done it is simply another way of referring to phosphoric acid. They are exactly the same compound with the same chemical formula. The "ortho" is simply referring to the fact that none of the hydrogens have been displaced (removed) from phosphoric acid (H_3PO_4). When the hydrogens are displaced to form phosphate compounds such as the salts of phosphoric acid there can be many phosphate salts produced and these may also be referred to as orthophosphates (e.g., sodium phosphates or sodium orthophosphates, potassium phosphates or potassium orthophosphates, calcium phosphates or calcium orthophosphates, etc.).

The quantity of each form of phosphorus (i.e., MCP and DCP) present in the final mixture can be predetermined by the manufacturer by controlling the amount of limestone used to react with phosphoric acid. This allows for the production of MCP and DCP which is familiar to the animal feed industry. The more limestone used in the reaction the more DCP will be formed and decreasing the amount of limestone will result in more of the MCP form. Normally, companies that manufacture feed-grade phosphates have high quality control standards and the guaranteed phosphorus and calcium contents of their products are very consistent from one batch to the next.

Reaction I (In this reaction Dibasic Calcium Phosphate (DCP) is formed)

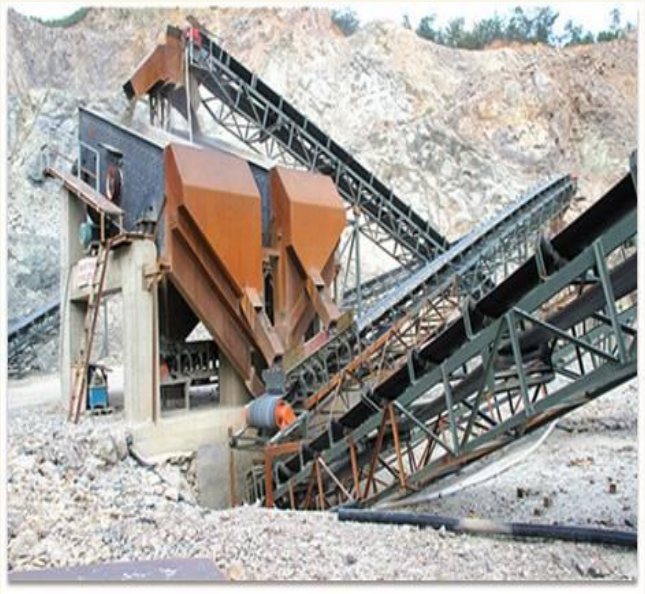
$$CaCO_3 \text{ (Limestone)} + H_3PO_4 \rightarrow CaHPO_4 + 2H_2O + CO_2$$

Reaction II (In this reaction Monobasic Calcium Phosphate (MCP) is formed)

$$CaCO_3 \text{ (Limestone)} + 2H_3PO_4 \rightarrow Ca(H_2PO_4)_2 \cdot H_2O + CO_2$$

In reaction I, the end product, $CaHPO_4$ contains Ca bound to the P in the dibasic ($HPO_4^{=}$) form and in reaction II the end product is $Ca(H_2PO_4)_2$ with one water of hydration and Ca is binding to two P in the monobasic ($H_2PO_4^{-}$) form.

Note: In each reaction above the phosphate compound that was produced contains only one calcium atom. Although the manufacturers of these feed-grade phosphorus products control the production variables in the reaction vessel, due to reaction kinetics there is not complete conversion to only one product (i.e., all MCP or all DCP). Therefore, because of this, in the production of MCP there exists some DCP in the final mixture. Similarly, in the production of DCP there exists some MCP.



The actual composition of the final feed-grade phosphate source (i.e., the total amount of MCP and DCP) is influenced by variables such as the ratio of limestone to phosphoric acid, concentration of phosphoric acid, temperature and the purity of the raw materials. However, the total concentration of phosphorus in the final feed-grade phosphate product will always be determined by the initial concentration of H_3PO_4 used in the manufacturing process.

As discussed previously, when purchasing MCP or DCP the buyer knows the minimum amount of phosphorus in the product, but not the exact form of phosphorus (i.e., (MCP or DCP). This is because the exact amount of each form in which the phosphorus exists is subject to some variability dependent on the degree of quality control implemented by the manufacturer. For instance, in a feed-grade phosphorus product such as DCP containing a guaranteed minimum phosphorus concentration of 18.5%, it is possible for the product to contain 20 to 50% of its phosphorus in the monobasic ($H_2PO_4^{-}$) form and 80 to 50% of its phosphorus in the dibasic ($HPO_4^{=}$) form. Also, for a product that is guaranteed to contain 21% phosphorus, such as MCP, there may be from 60 to 90% of the phosphorus in the $H_2PO_4^{-}$ form and 40 to 10% in the $HPO_4^{=}$ form. In either case, regardless of what form of phosphorus predominates in the final mixture of the feed-grade product, the total percentage of the two forms (MCP and DCP) must always equal 100%.

TRICALCIUM PHOSPHATE

A molecule of tricalcium phosphate (TCP) is totally different from MCP and DCP because it has all three hydrogens displaced from phosphoric acid [i.e., $Ca_3(PO_4)_2$]. This feed-grade product, which is considered a calcium orthophosphate, should actually be referred to as tribasic calcium phosphate. However, as with MCP and DCP, it is not common for someone to use the word "basic" when discussing TCP even though it is more "chemically" correct to do so. Another name which is commonly used when referring to TCP is "defluorinated phosphate" and this product must contain a phosphorus to fluorine ratio of, at least, 100:1 in order to be legally classified as being "defluorinated". A majority of the TCP produced in the United States as well as other countries is used in the diet of broilers because of several additional benefits associated with its use in the diet other than its content of phosphorus and calcium. These "bonus" benefits which are associated with TCP will be the topic of discussion in the next issue of Milwhite's Journal.

The majority of the TCP available to the animal feed industry is manufactured by mixing raw rock phosphate with phosphoric acid and soda ash (sodium carbonate, Na_2CO_3). This mixture is heated in a kiln at a very high temperature (~ 1300 to 1500 ºC). Raw rock phosphate is mined directly from the earth and may contain 13 to 14% phosphorus and 3.5 to 4.0% fluorine. Since raw rock phosphate contains such high concentrations of fluorine it is not suitable for direct use by the animal feed industry. The fluorine is driven off by the high temperature and the resulting product that contains an acceptable fluorine concentration is cooled, ground, screened and bagged. Normally, TCP contains approximately 17% phosphorus and because sodium carbonate is used in the manufacturing process TCP also contains sodium that nutritionists consider when they are formulating feeds.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

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UNDERSTANDING FEED-GRADE CALCIUM PHOSPHATE SOURCES
"TRI-CALCIUM PHOSPHATE"
(MORE THAN JUST A SOURCE OF PHOSPHORUS)

INTRODUCTION

In the last issue of Milwhite's Journal it was mentioned that tricalcium phosphate (TCP), commonly referred to as defluorinated phosphate (DFP), is associated with additional benefits when selected as a feed ingredient which go far beyond its contribution of P and Ca to an animal's diet. It is because of these benefits that TCP is preferred by many poultry nutritionists as the source of P in broiler diets. Since all of the TCP produced world-wide originates from P deposits on the earth's surface, it is appropriate to present a brief explanation of the origin of all P on our planet. All of the P on the earth's surface originated from igneous rock deposits formed when molten rock (lava), from volcanic activity, solidified. Following hundreds of millions of years of geologic exposure to different types of weathering and transport over the earth, the P in these original deposits ended up in today's world-wide commercial phosphate deposits mostly in the form of the calcium phosphate-rich mineral known as apatite, as well as in marine, river and other P rich rocks. It has been estimated that approximately 80% of the world-wide commercially mined phosphates are used as agricultural fertilizers and 15% are used in detergents, industrial products and food additives. The remaining 5% of the phosphates are used to produce feed-grade phosphate supplements for use in diets throughout the animal industry. Of this 5%, approximately 4.5% (i.e., 90%) is used to manufacture monocalcium and dicalcium phosphate. Therefore, only about 0.5% (i.e., 10%) of the world-wide phosphate being mined each year is used in animal feed as TCP.

ADDITIONAL BENEFITS OF USING TCP AS A DIETARY PHOSPHORUS SOURCE

All of the major commercially available high quality phosphate sources supplied to the animal feed industry are rigorously monitored by the manufacturers to ensure that they meet quality control standards. This provides assurance to the feed industry that the bioavailability of the P in the final product is high. Competent nutritionists are concerned about the bioavailability of P and other vital minerals in these phosphate supplements as well as the concentrations of toxic elements such as As, Pb and Cd. Experienced nutritionists look beyond the positive bioavailability data and seek out other reasons to select one product over another. The following is a brief summary about notable properties of TCP that should be given important consideration by nutritionists when choosing a phosphate source for use in diet formulation. Generally, the guaranteed minimum analysis of P, Ca and Na in high quality feed-grade TCP is reported as 18, 32 and 5%, respectively, with a P:F ratio of at least 100:1 which is required when a product is legally labeled as being "defluorinated". Also, the relative bioavailability of the P in TCP is normally reported to be above 93% and this value, or a slightly higher value, is used when formulating feeds.

DFP IMPROVES THE EFFICIENCY OF PELLETING: A major expense in the manufacture of animal feed is the high energy cost, which is especially true for pelleted feed. In 1981, Dr. Keith Behnke (Professor Emeritus, Kansas State University, USA) reported, in the Feedstuffs reference presented below, results of several replicated controlled pelleting experiments designed to identify the true differences that existed in the ability of various feed-grade phosphate sources to enhance the pelleting process. A significant increase in the production rate and energy efficiency resulted from the use of feed-grade DFP products instead of the monocalcium and dicalcium phosphate products. Dr. Behnke estimated that the use of a DFP product would result in a 20 to 30% decrease in time required to produce pellets and a savings of as much as 12% in energy consumption. Since DFP is a product manufactured from ground raw rock phosphate mined directly from the earth, Dr. Behnke concluded that the improved efficiency in time and energy is a result of the abrasive physical properties of DFP and this was the major factor responsible for maintaining a clean die-hole as the conditioned feed was being forced through the pellet die.

THE SPACE SAVING CONTRIBUTION OF Ca and Na in TCP: Nutritionists are very concerned with space limitations when formulating diets, especially for broilers and pigs because of the nutrient/energy dense diets they require. This is especially true in countries where energy sources are expensive. In this respect, the significance of TCP as a P source is easily realized when the computer considers TCP's contribution of Ca and Na to the diet. In this case, the computer formulation program will select and use less of the cheaper limestone which is to be used only as a source of Ca. By using TCP and removing some of the limestone, which is 38% Ca in the form of $CaCO_3$, provides more formulation space for nutrients and energy. The reason for this is because 62% of the weight of $CaCO_3$ is "CO_3" which is not contributing nutritionally to the diet and contributes greatly to energy/nutrient dilution. The same can be said for the bicarbonate in feed-grade sodium bicarbonate which is reported to contain 27% Na and 73% bicarbonate. The contribution of Na from TCP also allows for the use of less salt (NaCl) which contains 40% Na and 60% Cl and is important when nutritionists desire to minimize Na and/or Cl intake. For instance, it is a common practice in the turkey industry to use sodium bicarbonate in the diet in order to lower Cl which is being furnished by salt and thus, help prevent loose droppings and wet litter. Here is an example of where the Na contributed by using DFP lessens the cost of the diet by allowing for the use of less sodium bicarbonate which is more expensive. There is no dietary dilution when using TCP as a P source because the Na and the Ca are nutritionally valuable.

At times when it is desirable to improve performance by increasing dietary Na without increasing Cl, such as when the ionophore coccidiostat like monensin is used, the contribution of Na in DFP is a valuable asset to the nutritionist.

OTHER SITUATIONS WHERE DFP CONTRIBUTES TO BETTER POULTRY PERFORMANCE: Acidity (acidosis) is not promoted when DFP is used as the P source in a diet. This is important because there are various situations in egg-type and meat type poultry where deviations from a normal acid-base balance leads to metabolic complications related to vital processes involved in growth, immunity, bone formation, egg production, egg shell quality and survival during heat stress. The following is only a brief mention of several situations which serve to illustrate how using DFP assists in overcoming many of the negative impacts that acidosis may have on bird performance. In body fluids the electrolytes Na^+ and K^+ are associated with a rise in pH, whereas, Cl^- is associated with lowering pH. Reports in the scientific literature and from flocks of birds in the field document that excess Cl is associated with loose droppings (wet litter), poor egg shell quality and leg problems, especially tibial dyschondroplasia in broilers. Also, a major contributing factor related to a higher incidence of sudden death syndrome and ascites in broilers is excessive intake of Na and Cl. Therefore, it is not surprising why nutritionists would decide to lower dietary salt in this situation and use DFP to supply some of the Na. It is also important in such situations to consider all dietary contributors of Cl, such as choline chloride and L-lysine•HCl, in order to alleviate Cl related problems. Acidosis is also promoted when specific dietary components are metabolized in the body. Metabolic acidosis occurs as a result of feeding diets containing an excess of the monobasic form of calcium phosphate [$Ca(H_2PO_4)_2$] which happens when feed mill mixing errors occur. Metabolic acidosis is also related to sulfuric acid being formed as the divalent anionic sulfate radical ($SO_4^=$) is metabolized as a result of consuming the sulfate form of minerals, methionine and cysteine as well as the sulfur arising from consumption of canola meal. Therefore, lowering Cl by removing some of the salt "opens the door" for DFP to be used in the diet.

SUMMARY

From the above discussion it is obvious that along with being an excellent dietary P source there are additional benefits attributed to DFP which are directly related to its Ca and Na content. Along with its space saving advantage, anytime a nutritionist decides to increase Na or to lower the Na and CL content in order to minimize problems, as well as, promote better performance, DFP is able to play an important dietary role.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.
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UNDERSTANDING FEED-GRADE CALCIUM PHOSPHATE SOURCES
"HIGH DIETARY LEVELS OF MONOBASIC OR DIBASIC PHOSPHATE AFFECT LAYING HENS DIFFERENTLY"

INTRODUCTION

This is the last in a series of four issues of Milwhite's Journal dedicated to understanding more about monobasic calcium phosphate (MCP), dibasic calcium phosphate (DCP) and tribasic calcium phosphate (TCP) which are high quality feed-grade phosphate sources used by the world-wide animal industry. In 1994 Dr. K. Keshavarz, a faculty member in the Department of Animal Sciences at Cornell University (USA), reported results of several experiments with commercial egg-type laying hens that indicated they responded quite differently to high levels of P when the phosphate was in either the monobasic (H2PO4-) or dibasic (HPO4=) form. He attributed this difference in response to the strong acidic properties of the monobasic form of phosphate compared to the less acidogenic dibasic form. In his experiments Dr. Keshavarz formulated the laying hen diets using purified reagent-grade sources of these forms of phosphate [i.e., monobasic calcium phosphate monohydrate, Ca(H2PO4)2•H2O) and anhydrous or di-hydrated dibasic calcium phosphate, CaHPO4 and CaHPO4•2H2O, respectively]. , however, he did not use any commercially available feed-grade phosphate sources. Therefore, being familiar with this research, in 1997 Miles and Oelfke at the University of Florida, Gainesville, FL (USA) fed high dietary concentrations of P to laying hens using a feed-grade source of monobasic calcium phosphate and reported similar findings as those of Keshavarz (1994). Their data indicated that feeding a diet containing a high concentration of P supplied from a feed-grade source of monobasic calcium phosphate resulted in a decrease in laying hen performance. No decrease in performance resulted when the same dietary P concentration was supplied by a reagent-grade source of dibasic calcium phosphate. The following is a discussion of the laying hen experiment reported by Miles and Oelfke (1997).

EXPERIMENTAL APPROACH: Prior to the experiment conducted by Miles and Oelfke, these researchers surveyed the feed industry and purchased several commercial sources of feed-grade phosphate and had them subjected to X-ray diffraction analysis in order to document the exact form of phosphate present. The analysis was conducted by the Florida Industrial and Phosphate Research Institute located in Bartow, Florida (USA). From all of the feed-grade sources tested, a feed-grade source was selected that contained approximately 93% of its phosphate in the monobasic form. Also, one feed-grade source of tribasic calcium phosphate (TCP) was selected. A corn soybean meal basal diet was formulated to contain a concentration of 0.55% total P with 0.2% of the total P being supplied to the diet by a feed-grade source of dibasic calcium phosphate with the corn and soybean meal supplying 0.35%. Along with the basal (control) diet, four additional diets were formulated to contain 1% supplemental P supplied from reagent-grade dibasic calcium phosphate and monobasic calcium phosphate as used by Keshavarz (1994), feed-grade monobasic calcium phosphate and feed-grade tricalcium phosphate. This provided the five experimental diets, four of which contained 1.55% total phosphorus (Table 1). All diets contained the recommended concentrations of calcium, other nutrients and energy which met the bird's requirement for their age. Each diet was fed to 5 replications of 5 individually caged laying hens in an open-type house. The hens had ad-libitum access to their experimental diets and water during the entire 28 day-experimental period.

EXPERIMENTAL RESULTS: Within 24 hours after having access to their experimental diets there was a significant ($P < 0.05$) 60% decrease in feed consumption by the hens eating the high-P diet containing the phosphate supplied from either reagent-grade or feed-grade monobasic calcium phosphate. A low average feed intake by hens in these two experimental groups continued throughout the entire experimental period (Table 1). The immediate decrease in feed intake observed in this experiment was also observed by Keshavarz (1994) in hens fed high P from reagent-grade monobasic calcium phosphate. In his experiment, after 7 days the average daily feed intake was 107 grams whereas, hens fed the monobasic form averaged only 29 grams of feed intake each day. It is not known whether the decrease in feed intake which occurred as a result of consuming the diet containing the high concentration of P in the monobasic form was a result of poor palatability because the hens did not prefer the diet due to its acidogenic nature or whether the decrease in feed intake was a result of metabolic acidosis, as proposed by Keshavarz (1994). There was no decrease in feed-intake, egg production or overall laying hen performance resulting from hens consuming the diet containing high P in the dibasic form of calcium phosphate or from tribasic calcium phosphate (Table 1). Since X-ray diffraction analysis indicated that none of the phosphate in tribasic calcium phosphate was in either the monobasic or dibasic form, the normal feed intake by these hens was expected. Keshavarz also reported that feeding the dibasic form of phosphate did not affect hen performance, even when this form provided a supplemental P level in the diet of as high as 2.41%. In contrast, hen performance was seriously impaired when a supplemental P level of 1.02% was supplied in the monobasic form.

SUMMARY

As pointed out by Keshavarz (1994), depending on processing conditions such as the ratio of limestone to phosphoric acid, concentration of phosphoric acid, purity of raw materials, temperature of processing, etc., the percentage of monobasic and dibasic forms of phosphate can vary considerably in commercial feed-grade calcium phosphate sources used in the animal feed industry. Normally, companies that manufacture these feed-grade phosphates have strict quality control standards so that the ratio of each form of phosphate is usually known and kept somewhat constant from batch to batch of their product. Even though the monobasic form of phosphate is known to promote metabolic acidosis, no problems in performance of laying hens should be anticipated when this form is present in a feed-grade phosphate that is being used at normal dietary levels to meet the P requirement of the birds. No nutritionist would ever deliberately formulate and feed laying hens a diet containing such a high concentration of P which was used in the experiments mentioned above. However, when a diet unknowingly contains an unwanted high P concentration, such as when a feed mill mixing error occurs, major problems with feed intake and performance should be anticipated when the majority of the P in the feed-grade phosphate source is in the monobasic form, but not the dibasic form. The magnitude of the problem, as a result of the monobasic form, will be directly related to the actual amount of the monobasic form used in the diet and ultimately being consumed by the laying hens.

TABLE 1. EFFECT OF FEEDING LAYING HENS A HIGH CONCENTRATION OF PHOSPHORUS FROM DIFFERENT PHOSPHORUS SOURCES FOR 28 DAYS

PHOSPHORUS SOURCE	TOTAL DIETARY PHOSPHORUS (%)	FEED INTAKE (GM/DAY)	EGG PRODUCTION (% HEN-DAY)	EGG WEIGHT (GM)	HAUGH UNITS	EGG SHELL WEIGHT (GM)
CONTROL (DCP)[1]	0.55	109^{b}	85.7^{a}	63.1^{ab}	80.7^{a}	5.83^{a}
RG[2]-DIBASIC ($HPO_4^=$)	1.55	112^{ab}	83.3^{a}	64.5^{a}	83.6^{a}	5.74^{a}
RG[2]-MONOBASIC ($H_2PO_4^-$)	1.55	63^{d}	40.8^{c}	62.9^{ab}	80.5^{a}	5.57^{ab}
FG[2]-MONOBASIC ($H_2PO_4^-$)	1.55	67^{c}	50.0^{b}	61.9^{b}	84.0^{a}	5.29^{c}
FG[2]-TRIBASIC (TCP)[3]	1.55	113^{a}	82.7^{a}	63.8^{ab}	80.4^{a}	5.46^{bc}

[1]DCP = *DIBASIC CALCIUM PHOSPHATE*. ADDED AT 0.20% IN ALL DIETS WITH CORN AND SBM SUPPLYING 0.35% TOTAL P
[2,3]RG = REAGENT-GRADE, [2]FG = FEED-GRADE [3]TCP = TRIBASIC CALCIUM PHOSPHATE
[abc]MEANS IN COLUMNS WITH NO COMMON SUPERSCRIPT DIFFER SIGNIFICANTLY ($P<0.05$)

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Science at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL USA

Miles, R.D. and E. Oelfke. 1997. Laying hens respond differently to high dietary phosphorus from commercial sources. Poultry Sci. 76 (suppl. 1:143).
Keshavarz, K. 1994. Laying hens respond differently to high dietary levels of phosphorus in monobasic and dibasic calcium phosphate. Poultry Sci. 73:687-703.

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MILWHITE INC.
JOURNAL

AUGUST 2016

IMPROVED MILBOND-TX®

"ASSISTING POULTRY NUTRITIONALLY IN THE PRESENCE OF MOLDS AND MYCOTOXINS"

INTRODUCTION

There have been tens of thousands of publications which have detailed the negative effects that mycotoxins have on survivability and performance of poultry and other animals since the term "mycotoxin" was first used in 1962 to describe the reason for the death of approximately 100,000 turkey poults near London England. During the past 60 years, because of the highly toxic nature of mycotoxins in humans and domesticated animals, a tremendous amount of fundamental information has been learned about the molds (fungi) that produce specific mycotoxins and their mechanisms of action which are directly responsible for their lethal effects at the cellular level. We now know that the mechanism of the most toxic mycotoxins is a direct interference with DNA, RNA and protein synthesis within all body cells. Since mycotoxins are produced by various species of fungi, one of the best ways to prevent mycotoxin contamination is to prevent the growth of molds on foodstuffs, especially grains, which are a major source of nutrients and energy required for mold growth. Controlling the moisture content of grains post-harvest is essential if mold growth and subsequent mycotoxin synthesis is to be prevented. Also, there are several products available to the animal feed industry which are very effective in preventing mold growth in stored grain and mixed feed. The most common of these products are the organic acids, especially propionic acid. The organic acids will only inhibit mold growth, but will not have any effect on the mycotoxins already present. Stopping any further mold growth is the first step that should be taken.

If mycotoxins are already present in animal feed there are numerous commercially available products that are beneficial in preventing the devastating effects of mycotoxins. One of the most common approaches today, which is used world-wide, to prevent the negative effects mycotoxins have on animal performance is to use mycotoxin binders. These binders are added to the feed and will adsorb (bind) specific mycotoxins and prevent their absorption into the body from the digestive tract. Previous issues of Milwhite's Journal have addressed the efficacy of various binders used by the animal industry, especially the safety and effectiveness of Milbond TX® against Aflatoxin B-1 (AFB-1). If molds and mycotoxins are present in feed there are certain nutritional recommendations which have been shown to be beneficial in assisting poultry in coping with them. A decrease in feed intake is common when mold(s) and/or mycotoxin(s) contaminate feed. In this case, a universal recommendation often followed by nutritionists is to simply increase the nutrient density of the diet to help restore nutrient intake. In such situations feeding high quality, highly digestible feed ingredients will assist the bird greatly. Bird performance may not be fully restored, but increased nutrient intake and consuming high quality ingredients will help the bird to cope with the mold and mycotoxins. Knowing specifically which mycotoxin is present in the feed is very valuable information since certain nutritional recommendations are more effective against specific mycotoxins. The following is a brief summary of the nutritional recommendations that have been reported in the scientific literature as being beneficial, especially to poultry.

MOLD GROWTH AND RECOMMENDATIONS: Molds are heterotrophic organisms, just as all animals, protozoa and most bacteria are heterotrophic. Heterotrophs are not able to synthesize their own carbon-based food as autotrophs (i.e., plants) are able to do. Therefore, heterotrophs require preformed complex organic molecules present in plants and animals, to supply their nutrients and energy. A result of mold growing on grain is lower crude protein (CP) and metabolizable energy (ME) values of the grain and nutritionists must take this into consideration when formulating diets. Data collected with grain (especially corn) moderately infected with mold have shown that contaminated grain contains approximately 5% less protein and 10% less ME. For example, if uncontaminated corn had a value of 8% CP and 3,300 Kcal ME/Kg for poultry the values, because of mold damage, would be lower (i.e., 7.6% CP and 2,970 Kcal/Kg ME). Adjusting the computer program's ingredient matrix CP and ME values for the grain will assist the nutritionist in meeting their requirement for poultry. Of course, experienced nutritionists would more than likely use their own values for adjusting the amount of a specific grain's CP and ME.

Recently, (Chen et al. 2016, reference cited below) presented data showing that a high protein diet (26% CP) fed to broiler chicks from hatch to 20 days of age was able to completely prevent the detrimental effects that feeding a diet containing 1.5 mg/kg AFB-1 had on performance, nutrient digestibility and gut health. Feeding a diet containing 16 or 22% CP did not overcome the detrimental effects of AFB-1. Even though previous studies have shown that supplementing high protein and certain amino acids are beneficial to broilers fed diets containing AFB-1, this intensive study provided more detailed data on how a high protein diet assisted broiler chicks in overcoming the detrimental effects that AFB-1 had on overall intestinal anatomy and function, intestinal transporters, gut barrier/tight junction integrity, components of serum biochemistry, nutrient digestibility and breast meat yield.

OTHER RECOMMENDATIONS: When AFB-1 is the dietary contaminant an effective recommendation which will assist the bird nutritionally to minimize damage caused by AFB-1 is to supplement the diet with more synthetic methionine (e.g., 120-150% of requirement). Extra methionine will stimulate the synthesis of cysteine which is an amino acid essential for synthesis of the tri-peptide known as "glutathione (GSH)" which contains cysteine, glycine and glutamic acid. In the liver GSH binds with a very toxic metabolite of AFB-1 known as "AFB-1-8, 9 epoxide which is highly reactive and responsible for causing cellular damage at the level of DNA and RNA.

The GSH/epoxide complex forms "mercapturic acid" which is non-toxic and eventually excreted via urine and bile.

Supplementing 0.1% more choline to poultry diets has been shown to be effective with AFB-1 contamination. The extra choline will assist in moving lipid from the liver since AFB-1 is a hepatotoxin that injures the liver and interferes with lipid transport from the liver. Decreasing the lipid content of the diet when the fusarium toxin, diacetoxyscirpenol (DAS), is present has been shown to be beneficial in decreasing the absorption and subsequent negative effect on performance of this mycotoxin since it is lipid soluble. However, it is important to have some lipid in the diet since feeding a fat-free diet has been shown to depress the activity of certain liver enzyme systems that are responsible for detoxification of toxic compounds such as mycotoxins. The mechanism involved usually involves converting non-polar toxicants (i.e., mycotoxins) into more polar compounds which are more easily excreted from the body. These enzyme systems, along with other vital systems should not ever be limited from functioning at their full potential due to a lack of nutrients. It is believed, by researchers that this is why high quality, highly digestible protein sources such as fish meal have been shown to be beneficial in activating the protective enzyme systems that detoxify mycotoxins and help remove them from the body.

Using a vitamin/mineral stress package in the water and using specific antioxidants such as vitamins A, E, C is very beneficial during aflatoxicosis since AFB-1 induces oxidative stress in liver and other vital organs. Vitamin C is especially important in helping to maintaining the immune system. Selenium is also an essential part of antioxidant systems in the body and supplementing it to the diet at the highest legal amount permitted in the U.S. (i.e., 0.3 ppm) is beneficial. Fusarium mold interferes with the utilization of copper and thiamine. Supplementing 100 ppm Cu and 5 to 10 ppm thiamine during fusarium toxicosis has been shown to be beneficial and 200 ppm Cu has been reported to decrease the incidence of tibial dsychondroplasia caused by another Fusarium mycotoxin known as "fusarochromanone ". Supplementing the diets with 100 and 200 ppm Cu has also been shown to beneficial when less than 1 ppm AFB-1 is present.

SUMMARY

All of the nutritional recommendations mentioned above were taken from various reports in the scientific literature during the past 50 years that have been shown to be beneficial to poultry and other animals in minimizing the damage that molds and mycotoxins have on their performance and survivability.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Services at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, University of Florida, Gainesville, FL, USA.

Chen X., K. Naehrer, and T.J. Applegate. 2016. Interactive effects of dietary protein concentration and aflatoxin B-1 on performance, nutrient digestibility, and gut health in broiler chicks. Poultry Sci. 95: 1312-1315

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MILWHITE INC.
JOURNAL

SEPTEMBER 2016

IMPROVED MILBOND-TX®

"IMPROVED MILBOND-TX® PROMOTES INTESTINAL HEALTH"

INTRODUCTION

Serendipity" is often defined as making a beneficial unexpected discovery while investigating and searching for other things. Such was the case a decade ago at the University Of Florida (USA) while researchers were investigating the safety of Improved Milbond-TX (IMTX) when added to broiler and laying hen diets at higher than recommended concentrations. During the broiler studies researchers reported that excreta of birds fed a diet containing IMTX had less moisture than excreta from control birds fed no IMTX. Also, the "dryness" of the excreta could be easily identified visually by all individuals who were asked to evaluate the excreta for dryness. At the end of the study several birds from each dietary treatment were killed and the intestinal tract was opened longitudinally along its entire length. Visual observations of the tract's inner surface indicated it was extremely healthy and looked as if mucus was more abundant than that observed in the tract of control birds. This observation of a very healthy tract that seemed to have more mucus was reported by the researchers to Milwhite, Inc. along with their final research report of the IMTX safety studies. Thus, this simple unexpected observation prompted Milwhite, Inc. to initiate research to determine whether IMTX was indeed promoting better intestinal health. The three published articles listed below provided the data in 2012, 2013 and 2014 from the University of Illinois (USA) that clearly documented, respectively, that IMTX was, indeed, supporting intestinal health by alleviating diarrhea of weaned pigs challenged with a pathogenic E. coli, increasing intestinal goblet cell size and number thus, enhancing intestinal barrier integrity of pigs challenged with *E. coli* and stimulating positive changes in goblet cell function in broiler chicks challenged with *Salmonella enterica serovar Typhimurium*.

MUCUS AND INTESTINAL HEALTH

Goblet cells are mucus producing cells that are found all along the intestinal tract in close association with other cells located on each villus. Mucus has numerous essential functions, but one of the most important is its ability to serve as an intestinal barrier as it protects against fungi, bacteria and viruses as well as noxious agents that might possibly be in the intestinal lumen. Mucus is one of the immune system's first lines of defense associated with the intestinal tract since anything consumed must come into contact with mucus. Mucus also is the location of a major protective immune system antibody known as IgA that neutralizes bacteria trapped within the mucus. The functional properties of mucus are associated with its thick, slippery, slimy, viscous, gel-like physical characteristics that are responsible for its ability to coat the epithelial surface of the intestinal tract. The gel-like nature of mucus is a property resulting from the many sugars in its structure. Simply, mucus is a complex composed of a protein core (backbone) to which individual sugars is attached. This complex is also associated with inorganic salts within its structure. Thus, this complex is commonly referred to as a "glycosylated protein" (i.e., glycoprotein) known as "mucin" and there are several different glycoproteins (mucins) associated with mucus. It is the sugars that are coating (surrounding) the protein core that are responsible for the high water holding capacity since they attract water molecules. Thus, mucus lubricates the intestinal surface and protects the underlying epithelial cells. Mucus is not static and is constantly being sloughed from the surface of the intestine along with the numerous bacteria, viruses and any noxious substances trapped within the mucus. When new mucus replaces the sloughed mucus this provides a very important protective mechanism for the intestine since mucus is constantly covering and protecting the underlying epithelial cells from unwanted attachment of bacterial pathogens.

RESEARCH WITH CLAYS

Of the three publications cited below involving IMTX, the publication of Almeida et. al., (2014) is the most significant because it provides data which possibly explains the mechanism(s) of how a specific smectite clay promotes enteric health which was documented in the 2012 and 2013 publications from their laboratory. In their 2014 study, these authors specifically designed *in-vivo and in-vitro* experiments with the objective of testing the beneficial effects of using three different clays in diets of young broiler chicks that had been challenged with pathogenic Salmonella. Each clay was added to the diet at a concentration of 0.3% and identified as smectite A, smectite B and zeolite. Smectite A was IMTX. Results indicated that each clay was able to restore the loss in growth performance of the Salmonella-challenged chicks.

However, only the smectite A clay was able to promote changes in goblet cell function and size and be a possible mechanism of action of this specific smectite clay (IMTX) in promoting a strengthening of the intestinal mucosal barrier.

Synthesis of a goblet cell's proteins that are associated with intestinal barrier protection first requires activation (turning on) of protein encoding genes. Almeida and coworkers (2014) reported that IMTX was the only clay that activated genes associated with the synthesis of a goblet cell protein specifically responsible for maintaining the intestinal mucosal defense barrier. This protein is known as RELMß (RELM-beta). Also, IMTX promoted the synthesis and secretion of another protein known as MUC2 (Mucin 2) which is quantitatively the most important of the mucin proteins responsible for intestinal barrier health. As pointed out by these authors, these data provide evidence that some, but not all, clays help improve intestinal barrier function of chicks during an enteric infection. From the data collected in numerous previous experiments and those data reported by the authors cited below, it should be obvious that IMTX is a unique clay-based product that falls into the category as being classified as a "privileged" feed additive. IMTX has proven to be a safe effective mycotoxin binder in the lumen of the intestinal tract while at the same time provides direct benefits to intestinal health by strengthening the mucosal defense barrier. In the next issue of Milwhite's Journal the authors will explain, in an easy to understand manner, the cellular mechanisms involved in how IMTX is promoting intestinal health by promoting gene expression and synthesis of the MUC2 and RELMß mucin proteins.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Services at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, Univ.of Florida, Gainesville, FL, USA.

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Almeida, J.A.S., Y. Liu, M. Song, J.J. Lee, H. R. Gaskins, C. Wolfgang Maddox, O. Osuna and J.E. Pettigrew. 2013. Escherichia coli challenge and one type of smectite alter intestinal barrier of pigs. J. Animal Science and Biotechnology 4:52.

Almeida J.A.S., N.P. Ponnuraj, J.J. Lee, P. Utterback, H.R. Gaskins, R.N. Dilger, and J.E. Pettigrew. 2014. Effects of dietary clays on performance and intestinal mucus barrier of broiler chicks challenged with Salmonella enterica serovar Typhimurim and on goblet cell function in vitro. Poultry Science 93:839:847.

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MILWHITE INC.
JOURNAL

OCTOBER 2016

IMPROVED MILBOND-TX®

"IMPROVED MILBOND-TX® PROMOTES INTESTINAL HEALTH"
"UNDERSANDING THE MECHANISM OF ACTION"

INTRODUCTION

In the previous issue of Milwhite's Journal (September, 2016) the research conducted by Almeida et. al., (2014) at the University of Illinois (USA) was discussed and documented the fact that of two smectite clays and a zeolite that were tested only one smectite clay was able to promote intestinal health. That clay was IMTX. These authors provided data that showed all three clays restored the lost performance in chick body weight and feed intake resulting from a *Salmonella* challenge. However, of the two smectite clays tested only IMTX was reported to initiate changes in goblet cell function by stimulating mucus synthesis and release which strengthened the integrity of the intestinal mucus barrier. The purpose of this issue of Milwhite's Journal is to explain, in an easy to understand fashion, the reason why these investigators decided to select out and study specific components of the mucosal immune system when testing these clays. These components of the immune system, which are proteins, are considered to be among the most important proteins that are synthesized when the animal's intestinal tract is challenged by pathogenic microbes A full explanation of the details of their *in-vivo* and *In-vitro* experiments can be found in their publication cited below.

GOBLET CELLS AND ITS MUCIN PROTEINS

At this point, in order to facilitate a better understanding of the data collected and reported by these authors which explains how IMTX is promoting intestinal health it is essential to appreciate the difference between mucus and mucin. Mucus is the viscous gel-like fluid that is actually secreted onto the surface of the intestinal epithelium by the goblet cells associated with the intestinal villi. The gel-like nature of mucus is a property resulting from the numerous sugar molecules in its structure. Simply, mucus is a composite composed of a protein core (backbone) to which individual sugars are attached. This carbohydrate-rich protein complex also has inorganic salts associated with its structure. Thus, this complex is commonly referred to as a "glycosylated protein" (i.e., glycoprotein) known as "mucin" and there are several different glycoproteins (mucins) associated with mucus. It is the sugars that are coating (surrounding) the protein core that are responsible for the high water holding capacity of mucus since the mucins attract an enormous quantity of water molecules. In fact, some mucin proteins are composed of over 85% of these sugars and are responsible for mucus being composed of approximately 95% water. So, mucus is composed of mucins which are glycoproteins associated with inorganic salts that becomes very viscous after this composite attracts water.

GOBLET CELL SYNTHESIS OF SPECIFIC MUCINS

In order for goblet cells to synthesize their many mucin proteins there must be a mucin gene that encodes each protein. Each individual mucin protein has a specific defined function. Specifically, one of the most important mucin proteins is referred to as the MUC2 protein (i.e., MUCIN 2 protein) and the coding gene for this protein is the MUC2 gene. Even though there are different types of mucins produced in various locations within an animal's body, MUC2 is quantitatively the most important protein in intestinal mucus associated with epithelial barrier protection and this is one reason that Almeida et. al., (2014) were interested in this goblet cell protein. It has been estimated by numerous researchers that as much as 80% of the mucins produced by goblet cells are MUC2. This MUC2 protein is responsible for the viscus "net-like" structural property of the mucus secreted by goblet cells. Not only did Almeida and coworkers report on the goblet cell production of MUC2 protein, but they also reported data on how two other essential goblet cell proteins were influenced by different clays. These two proteins were TREFOIL FACTOR 3 (TFF3) and RESISTIN-LIKE MOLECULE BETA (RELM-ß). As with MUC2, being only one of several in the family of mucin proteins, TFF3 and RELM-ß are only one in a family of different TFF and RELM proteins produced by goblet cells. The reason why these two families of proteins were also a main focus of this study was because they have extremely important functions in maintaining integrity of the intestinal mucosal surface associated with "certain situations".

SYNTHESIS OF TREFOIL FACTOR 3

A situation where TFF3 protein is synthesized from its specific gene (TFF3 gene) in goblet cells occurs when there is a need for mucosal defense or a healing function in the intestine as a result of some insult. Also, there is evidence that TFF3 protein assists in stabilizing the entire mucus layer along with providing protection against ulcerative conditions in the intestinal tract which are common during pathogen colonization. For instance, whenever there is an injury or an ulcerative condition in the intestinal epithelial surface the TFF proteins act as "hormone-like messengers" and promote the migration of healthy cells in the region of the injury without promoting cell division. Thus, a mode of action of these TFF proteins is related to their ability to stimulate the migration of surviving cells from the edge of any damaged region of the intestine over the uncovered (denuded) area promoting the healing process. One might wonder why this family of intestinal proteins is referred to as the "Trefoil peptide family". The Latin word "Trifolium" (tri-"three" and folium-"leaf) refers to the genus of the legume family of plants that have leaves divided into three distinct areas called leaflets (i.e., common clovers). Since the proteins belonging to the Trefoil family have three distinct and identifiable "loops" in their structure this name reflects these three distinct structural loops or more commonly referred to as a protein having three distinct structural "domains". The TFF family of peptides is characterized by having six molecules of the sulfur containing amino acid, cysteine, in their structure.

This is of importance because as part of the protein structure these six cysteine molecules form three distinct intra-chain disulfide bonds which are responsible for forming and maintaining the unique three-loop structural domain configuration of the trefoil peptide family.

SYNTHESIS OF RESISTIN-LIKE MOLECULE ß

The third cysteine-rich goblet cell protein, RELMß, was of importance to these investigators since the chicks were challenged with Salmonella and RELMß is synthesized and released from goblet cells in situations where bacterial colonization occurs. Maintaining and stabilizing the mucosal defense barrier as well as helping to regulate and control intestinal inflammation following mucosal injury are also considered main responsibilities of this protein. RELMß has also been shown to upregulate the MUC2 gene so more mucus will be formed during infections and released from goblet cells and as a result goblet cells decrease in size. This hormone-like protein derives its name from another cysteine-rich protein called "Resistin" which is known to be produced by fat cells and was only discovered in 2001 at the University of Pennsylvania Medical School (USA). It was called Resistin (Resist-"Resistance" and in-"insulin") because it promoted insulin resistance when it was injected into mice. Insulin resistance occurs when muscle, fat, and liver cells do not respond properly to insulin and results in the absence of glucose absorption into the cells of these tissues and the beta-cells in the pancreas become hyperactive attempting to produce more insulin. Over time insulin resistance leads to Type-2 diabetes. Because this goblet cell-derived protein has also been shown to impair insulin function just like Resistin, it was named "Resistin-Like Molecule Beta" (RELMß).

RESEARCH FINDINGS

The importance of the data reported by these researchers investigating the smectite clay, IMTX, should be obvious. Their data, related to goblet cell number and size, supported the concept that of the three clays tested only IMTX was able to promote effects consistent with strengthening the intestinal mucus barrier. In their *in-vitro* experiment data indicated that no clay showed an ability to alter the gene expression of TFF3. However, only IMTX increased expression of the RELMß gene in the human colorectal cell-line, LS174T, selected for use in this study. Data collected on gene expression in these cells indicated that IMTX promoted more synthesis and release of the RELMß protein. In this study, all three clays were shown to have a beneficial effect in restoring the loss in chick body weight and feed consumption which resulted from the *Salmonella* challenge. However, these researchers mentioned that in certain cases only IMTX was the smectite clay that promoted positive benefits compared to the other clays tested. Importantly, they emphasized that just because a clay is a smectite does not mean that it will promote similar positive benefits as those observed with IMTX in this study.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Services at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, Univ.of Florida, Gainesville, FL, USA.

Almeida J.A.S., N.P. Ponnuraj, J.J. Lee, P. Utterback, H.R. Gaskins, R.N. Dilger, and J.E. Pettigrew. 2014. Effects of dietary clays on performance and intestinal mucus barrier of broiler chicks challenged with Salmonella enterica serovar Typhimurium and on goblet cell function in vitro. Poultry Science 93:839-847.

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MILWHITE INC.
JOURNAL

NOVEMBER 2016

IMPROVED MILBOND-TX®

"STRATEGIES EXIST FOR COPING WITH POOR QUALITY GRAIN: PART 1"

INTRODUCTION

In January, 1997 Dr. Orlando Osuna and Dr. Richard Miles were coauthors of an article published in a special issue of Feedstuffs, the weekly newspaper for agribusiness. Their article, cited below, was selected as the "feature article" in this special issue of Feedstuffs which was published during the week of the annual International Poultry Exposition in Atlanta, Georgia (USA) because it contained valuable information which could be used immediately by the world-wide feed and poultry industry about how to handle and cope with poor quality grain, especially grain contaminated with molds and mycotoxins. Since, this Feedstuffs article was so well received by the world-wide feed and poultry industries and the strategies defined and discussed in the article for dealing with poor quality grain remain relevant today, this issue and the next two issues of Milwhite's Journal are dedicated to presenting this valuable information to our clients. The information presented will make it evident how important it is for feedmill managers, nutritionists, quality control personnel, veterinarians and poultry production managers to work as a team to establish company guidelines for storing and segregating their incoming shipments of grain. In so doing, good quality grain can be protected and by utilizing the strategies outlined in the article, the negative effects that poor quality grain are known to have on poultry performance will be able to be minimized so that as much as possible of the bird's genetic potential for performance will be realized.

QUALITY CONTROL-THE FEEDMILL

The quality of raw materials used for feed production by poultry feedmills is of extreme importance because what birds eat can affect flock quality and the wholesomeness of a flock's meat and eggs. Most raw materials used as an energy and/or protein source in poultry diets are grown, harvested, processed and transported by someone not involved with the poultry industry. Therefore, the feed ingredient quality control component of a poultry operation's feedmill is an important first step in protecting the birds on the farm. No universal quality control program is best for all feedmills because each feedmill presents a unique management situation. However, some commonalities exist in all feedmills. One commonality is a lack of uniformity in the grain received regardless of its source. Consequently, the quality of the grain is variable with regards to its nutrient content and amount of physical, chemical and/or microbiological contamination. During the past 40 years, Drs. Osuna and Miles have had countless opportunities to visit feedmills and poultry production facilities throughout the world. They both agree that one of the most common problems that existed was related to grain and feed handling and making meaningful decisions when molds and mycotoxins were involved. In many instances they have encountered situations in which mycotoxins, at low concentrations, were present and there was no developed and/or implemented strategy to cope with them in a satisfactory manner. Most birds housed under commercial conditions are more commonly subjected to chronic low level exposure rather than acute high level exposure to mycotoxins.

ENEMIES OF GRAIN DURING STORAGE

There are numerous excellent articles written on how poor quality grain influences poultry health and performance. However, it is essential to understand that there are a multitude of factors that have an influence on the quality of grain once it is stored in silos. These factors cannot be considered alone because they are all interrelated. Without an understanding and an appreciation of these interrelationships it is nearly impossible for anyone to implement strategies to adequately control the deterioration of stored grains which is caused by molds. One of the major strategies to control grain deterioration during storage is to control the moisture in the grain. Moisture is the number one enemy of stored grain and proper grain drying is essential. If moisture is controlled, mold growth is controlled and mycotoxin production is usually not a problem, unless the grain contains mycotoxins that have been previously produced by the molds. Consequently, many feedmills focus the majority of their efforts in prevention of mold growth during grain storage. Even if the number one enemy of grain is known to be moisture, the role that insects and rodents play in causing mycotoxin and other quality control problems must not be overlooked. Insects and rodents lead to the production of "fines" (dust) by compromising the structural integrity of the grain kernel. Any decrease in kernel integrity will increase the possibility of eventual mold and/or bacterial contamination. Dust is known to be a food source for insects and rodents and a major source of salmonella contamination in feedmills and every attempt should always be made to control dust formation and its accumulation in and around feedmills. In addition, controlling rodents will decrease the likelihood of fire and dust explosions due to the damage that rodents cause with electrical wiring. Air quality is also enhanced when dust is controlled and controlling dust decreases respiratory problems which are known to occur in feedmill employees continually exposed to dust. Some of the best and most expensive mold preventive measures designed for stored grain often fail because an integrated pest control and sanitation program has not been developed simultaneously. Feed bin cleanliness should be monitored on a regular basis. Grain storage bins, their boots and all associated augers should be included as an essential component of a company's Hazard Analysis and Critical Control Point (HACCP) program. Immediate clean-up should always follow a feed and/or oil spill and unsanitary conditions around the feedmill should be rectified in a timely manner.

REQUIREMENTS FOR MOLD GROWTH

Molds are ubiquitous and prior to harvest there is presently no practical way to prevent their presence or the presence of their spores. So, there will always be a threat of mycotoxin production in grain prior to harvest when favorable environmental conditions exist that promote mold growth. This is why pre-harvest mycotoxin concentration varies in grains from year to year, especially when environmental stressors such as drought and insect damage are high. Often, a company does not know the geographic origin of their grain. Knowing the location would prove beneficial during the screening for certain mycotoxins. For example, it is known that aflatoxins tend to predominate in grain that is grown in warmer climates and has a higher oil content such as corn instead of wheat and oats since aspergillus thrives on high oil grains. Grain originating in cooler climates is usually associated with contamination with Fusarium toxins such as fumonisin, deoxynivalenol and zearalenone.

Mold growth is associated with five factors: a nutrient source (the grain), moisture (usually above 12%), warm temperature, oxygen and time. As molds grow, hair-like structures known as hyphae immediately cover and penetrate the grain. Digestive enzymes are released from the hyphae and the nutrients are broken down and absorbed and utilized by the mold. Molds are heterotrophs, just like animals, and require preformed organic compounds as an energy source. Therefore, the carbohydrate, fat and protein are able to be utilized for energy. This is why grain subjected to mold growth is associated with less metabolizable energy. Once grain arrives at a feedmill and is placed in a storage silo its quality begins to decline as long as the grain remains in storage. Grain quality does not improve during storage. The best that can be expected is for its quality to be maintained for as long as possible until it is used in feed formulation. The rate of deterioration will depend on grain management practices that a company has incorporated into their quality control program. Therefore, purchasing high quality grain is most desirable and minimizing its deterioration during storage should always be major objectives. In the next issue of Milwhite's Journal strategies will be discussed that have been shown to minimize the rate of grain deterioration during storage.

The information presented in this issue of Milwhite's Journal was compiled by Dr. Orlando Osuna, Director of Health Services at Milwhite, Inc. and Dr. Richard Miles, Professor Emeritus, Univ. of Florida, Gainesville, FL, USA.

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