ABSTRACT

WALTMAN, LINDSEY. Effect of Sequestering Agents on Aflatoxin in Milk of Dairy Cows Fed Aflatoxin-contaminated Diets. (Under the direction of Dr. L. W. Whitlow and Dr. B. A. Hopkins).

Three experiments (EXP) were conducted to determine the potential of experimental sequestering agents, clays or non-digestible yeast oligosaccharides, to reduce milk aflatoxin concentration in lactating Holstein cows consuming aflatoxin in their diet. All EXP included two periods in a randomized block design. Cows were fed an aflatoxin-contaminated total mixed ration (TMR) for both periods of all trials. During the first period, cows received no sequestering agents in the TMR, but agents were included in the TMR for the second period. EXP 1 and 2 consisted of two 7-d periods with 12 cows per treatment. Milk aflatoxin (AFM_1) concentrations were analyzed by HPLC for milk samples collected on d 5 to 7 and d 11 to 13. Two treatments in EXP 1 were: 1) control (no sequestering agent n=12), and 2) 100g/cow/day Lallemand n=12. Four treatments in EXP 2 were: control (no sequestering agent) n=12, 2) 10g/cow/day MTB-100[®] (2004) n=12 (Alltech, Inc., Nicholasville, KY), 3) 10g/cow/day MTB-100[®] 2006 n=12 (Alltech, Inc., Nicholasville, KY) n=12, and 4) 10g/day/cow Alltech experimental (Alltech, Inc., Nicholasville, KY) n=12. EXP 3 consisted of two 8 d periods and included 14 cows. Milk samples from d 4 to 8 and d 11 to 16 were analyzed for AFM_1 concentrations by ELISA. Three treatments in EXP 3 were: 1) control (no sequestering agent) n=4, 2) 10g/cow/daily MTB-100[®] 2006 (Alltech, Inc., Nicholasville, KY) n=5 and 3) 227g/cow/daily Astra-Ben 20A[®] (AB-20A[®]) (Prince Agri Products, Inc., Quincy, IL) n=5. For all EXPs, the percent differences in AFM_1 concentrations between

periods 1 and 2 were calculated. All percent differences were normalized using a correction factor that converted values for controls to zero. In EXP 1, the addition of a mixture of NYO-A and diatomite-montmorillonite resulted in a 5.2% numerical increase in AFM_1 concentration. In EXP 2, MTB-100[®] (2004), MTB-100[®] (2006), and Alltech experimental product resulted in 8.0%, 6.2%, and 9.5% numerical increases in AFM_1 concentrations respectively. In EXP 3, MTB-100[®] (2006) resulted in a 5.1% numerical decrease in AFM_1 concentrations, and AB-20A [®] resulted in a 60.4% significant decrease in AFM_1 concentrations. In summary, the AB-20A [®] in EXP 3 reduced AFM_1 concentrations however, there were no significant changes in AFM_1 concentrations in response to sequestering agents other than AB-20A[®].

Effect of Sequestering Agents on Aflatoxin in Milk of Dairy Cows Fed Aflatoxin-
contaminated Diets

by
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BIOGRAPHY

Lindsey Waltman was born in Bryn Mawr, Pennsylvania on May 20, 1983 to Mr. and Mrs. Joseph J. Waltman. She graduated from the Shipley School and began college at the University of Delaware in the fall of 2002. Lindsey received a B.S. degree in Animal Science with a concentration in pre-veterinary medicine in May of 2006. She entered the North Carolina State University Animal Science program in the fall of 2006 to pursue an M.S. degree in Animal Science and Nutrition. Following graduation Lindsey will begin a combined Doctor of Veterinary Medicine and PhD program at North Carolina State University.

ACKNOWLEGMENTS

DEDICATION

To my father with all my love, who inspired me to pursue my dreams.

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LITERATURE REVIEW

INTRODUCTION

Aflatoxins represent a group of mycotoxins produced primarily by Aspergillus *flavus* and Aspergillus *parasiticus*. Aflatoxins are carcinogens that routinely occur in feed grains. A. *flavus* commonly contaminates corn, peanuts and cottonseed. This fungus typically produces aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2), and varies greatly in toxigenicity. Aspergillus *parasiticus* is most prevalent in peanuts and produces aflatoxins G_1 (AFG_1) and aflatoxin G_2 (AFG_2), in addition to the B aflatoxins. In contrast to A. *flavus*, A. *parasiticus* generally produces high levels of aflatoxin. Aflatoxin is considered an unavoidable contaminant in which contamination can occur during all stages of growth, harvest, storage and feeding. Seventeen aflatoxin compounds have been isolated, however only four of these metabolites are currently of major concern. These compounds are AFB_1, AFB_2, AFG_1, and AFG_2. Of the aflatoxins, AFB_1 is found in the highest concentrations and is currently regulated by the Food and Drug Administration (FDA). The order of toxicity is as follows: $AFB_1 > AFG_1 > AFB_2 > AFG_2$ (McLean and Dutton, 1995). Aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) are hydroxylated metabolites and secreted in the milk of animals consuming aflatoxin. Aflatoxin M1 is 4-hydroxy aflatoxin B_1 and aflatoxin M_2 is 4-dihydroxy aflatoxin B_2. Shortly after AFB_1 contaminated feed is ingested, AFB_1 is hydroxylated and secreted in milk as AFM_1. The amount of AFM_1 secreted in milk from dairy cattle is approximately 1 to

2 % (1.7 % average) of the dietary AFB_1 level (van Egmond 1989). Cows fed AFB_1 contaminated feed can develop health problems such as hepatic carcinomas, immunosuppression, reproductive dysfunction, decreased milk yield and decreased feed intake. Humans can develop similar health problems when consuming food contaminated with either AFB_1 or AFM_1. Due to the carcinogenicity of AFB_1 and AFM_1, the FDA has set an action level of aflatoxin in feedstuff for lactating dairy cattle to a maximum of 20 ppb. The transfer of aflatoxin from feed to milk is of concern for two reasons: first, due to the consumption of milk and milk products by humans, especially infants and children. Secondly, milk containing concentrations of AFM_1 greater than 0.5 ppb is not permitted for use in human consumption as stated by the Food and Drug Administration (Code of Federal Regulations Part 109). Therefore, milk containing AFM_1 above the action level must be discarded, which can cause significant economic loss for the dairy producer. Certain feed additives and post-harvest strategies offer the potential to reduce the contamination of milk with aflatoxin.

This literature review is divided into the following sections:

1. Aflatoxin Metabolism
2. Metabolic Detoxification
3. Post-harvest Detoxification Strategy
   a. Mechanical Separation

b. Density Segregation

c. Thermal Activation

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AFLATOXIN METABOLISM

After ingestion, AFB_1 is absorbed through the small intestine and enters the heptaocyte, where biotransformation occurs. Biotransformation is important for the biological activity and disposition of aflatoxins. Aflatoxin biotransformation research primarily focuses on AFB_1 because of its toxicity and carcinogenicity. Also, AFB_1 is usually
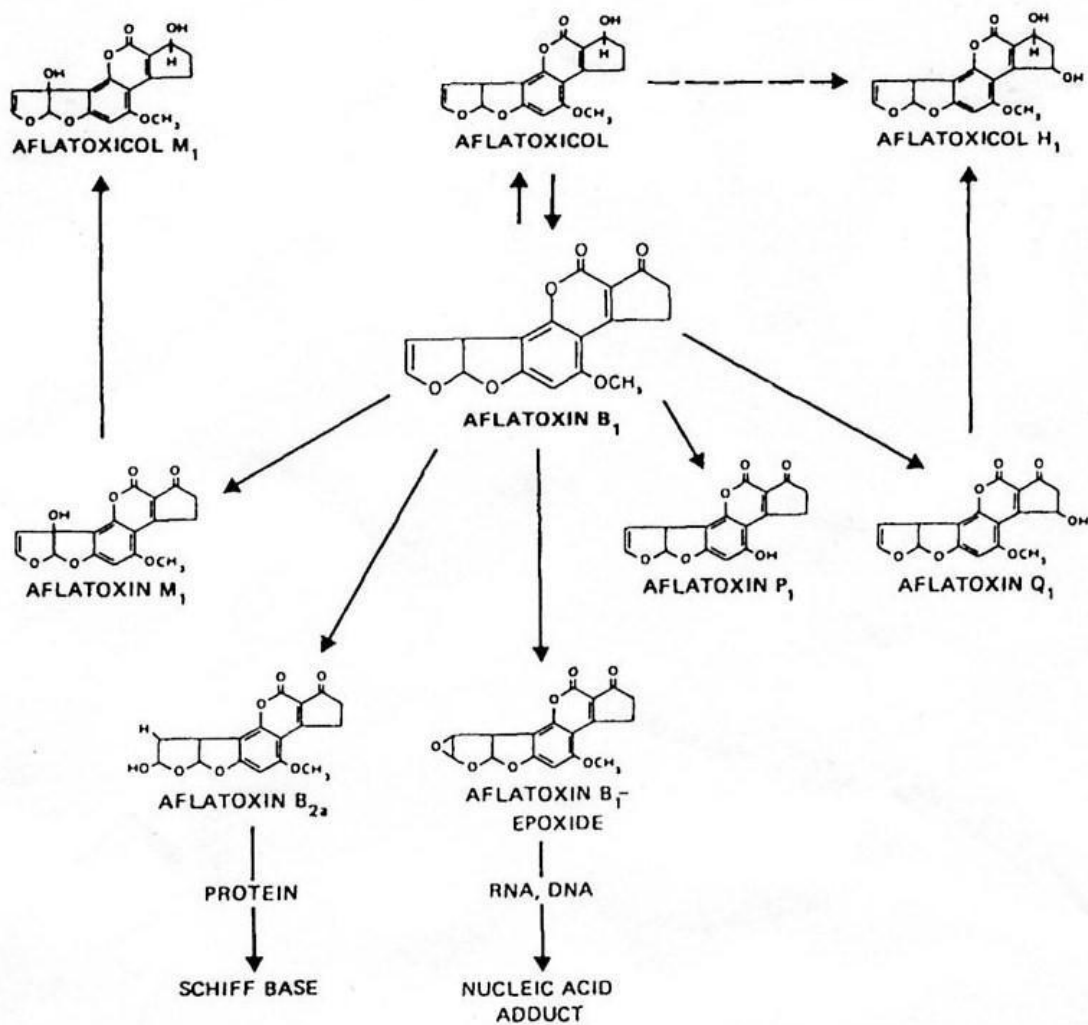
Figure 1. Metabolism of aflatoxin B_1 (World Health Organization, 1979).

found in the highest concentrations in contaminated feed. Biotransformation of AFB_1 can

lead to a variety of products through various pathways (Figure 1).

Once AFB_1 enters the cell there are two fates. AFB_1 can be oxidized to reactive epoxides or can be converted to hydroxylated metabolites. Conversion of AFB_1 to a reactive epoxide is called metabolic activation and is required for aflatoxin to exert its toxic and carcinogenic effects (Garner et al., 1972). AFB_1 is converted to the reactive AFB_1-8,9-epoxide (AFBO) through oxidation of the 8,9-vinyl ether bond. Subsequently, AFBO is transported across the plasma membrane; which is followed by activation through microsomal mixed function mono-oxygenases. Formation of the highly reactive AFBO requires cytochrome P450, NADPH and molecular oxygen (McLean and Dutton, 1995). AFBO can induce mutations by intercalating to DNA and forming an adduct with guanine moiety in the DNA (Smela et al., 2001). This intercalation of AFBO causes a Gà T transversion at codon 249 in p53 gene in liver, which may lead to hepatic carcinoma. This is thought to be the major reason for aflatoxin carcinogenecity (Railey et al., 1997). AFBO can also undergo spontaneous hydrolysis to form the AFB_1-8,9-dihydrodiol (AFB dihydrodiol). Both AFBO and AFB dihydrodiol can bind to proteins resulting in cytotoxicity. When AFB dihydrodiol binds to protein it forms an AFB-lysine adduct which can be detected in serum. In addition to AFBO, microsomal biotransformation results in the production of more polar metabolites such as aflatoxicol, aflatoxin Q_1, aflatoxin P_1, and aflatoxin M_1. The toxicities of the hydroxylated metabolites are usually less than the parent compound (Stoloff et al., 1972; Hsieh et al., 1974). This is also true for the mutagenic potencies (Coulombe et al., 1984). One study using trout to determine the carcinogenic potential of AFM_1 found that AFM_1 was

approximately 30% less carcinogenic than AFB_1 (Sinnhuber et al., 1974). Additionally, Hsieh et al. (1984) reported that AFM_1 was approximately 10% as carcinogenic as AFB_1 in rats (Hsieh et al., 1984).

METABOLIC DETOXIFICATION

After biotransformation, the reactive metabolite of aflatoxin can undergo metabolic detoxification. The primary pathway for detoxification in many species is through the conjugation of AFBO with glutathione (GSH), mediated by glutathione s-tranfersase (GST) (Eaton and Gallagher, 1994). A study conducted by Hayes et al. (1991) measured AFB_1-DNA adduct formation when animals were fed aflatoxin-contaminated diets. This study found that tissues with GST activity had decreased AFB_1-DNA adduct formation. There is wide variability in the susceptibility of animals to AFB_1. Studies have shown mammalian species susceptibility to AFB_1 hepatocarcinogenesis is highly correlated with the selectivity of GST isoenzymes towards AFBO (Lotlikar et al., 1984; Eaton and Ramsdel, 1994; Roebuck and Wogan, 1977). Smith et al. (1984) conducted a study to evaluate GST activity among different species. They reported GST activity expressed in bovine hepatocytes was much lower than that of rat hepatocytes. These findings are consistent with those of Eaton and Gallagher (1994) who reported a negative correlation of GST activity and concentrations of AFBO in rodents.

DETOXIFICATION STRATEGIES

Numerous strategies for detoxifying aflatoxin have been evaluated. Some have proven to be more effective and practical then others. The most effective route of decreasing aflatoxin is complete removal; however, this is not always economically feasible. Therefore, guidelines for the process of detoxifying mycotoxins have been established and are: (1) inactivate, destroy or remove the mycotoxin, (2) detoxification should not result in the deposition of toxic substances, metabolites, or by products in the food or feed, (3) retain nutrient value and feed acceptability of the product or commodity, (4) detoxification should not result in significant alterations of the products properties and if possible (5) destroy fungal spores. (Park et al., 1988). This review will evaluate post harvest strategies for detoxifying aflatoxin.

Mechanical Separation

Mechanical separation is a process based on identification of damaged kernels through variation in size, shape, color and visible mold growth. Aflatoxin-contaminated kernels can appear damaged, shriveled or discolored; hence a combination of sieving and electronic sorting can eliminate some of the contaminated kernels. After sieving and electronic sorting are employed, hand picking can be used to remove any contaminated

kernels that escaped the initial processes. Dickens and Whitaker. (1975), found a significant decrease in aflatoxin levels when both electronic sorting and hand sorting were used to decontaminate peanuts. Although aflatoxin concentrations may significantly decrease following mechanical separation, this method is not very practical due to the incomplete removal of mycotoxin contaminated grains (Natarajan et al., 1975).

Density Segregation

Density segregation involves the sorting and delineation of non-contaminated versus contaminated feed by flotation. Huff (1980) conducted a study in which aflatoxin contaminated corn was placed in vessels containing either water or sucrose solution. Buoyant corn was determined to be contaminated with aflatoxin. In a second study, Huff (1988) significantly reduced the level of aflatoxin by removing the buoyant and semi-buoyant corn from the water containing vessels. It was determined that semi-buoyant corn had the same level of aflatoxin as buoyant corn. Additionally, Huff (1989) found by increasing the density of the liquid used for segregation improved the segregation process resulting in the removal of more aflatoxin-contaminated corn. On the contrary, Stoloff (1972) found physical methods such as dry cleaning, wet cleaning, and density separation were not effective at lowering aflatoxin content in contaminated corn.

Thermal Activation

Aflatoxin is a heat stable mycotoxin that it is not completely destroyed by heat treatments, such as boiling water and autoclaving (Christensen 1977). However, Marth and Doyle (1979) found that partial destruction of aflatoxin can accomplished by oil roasting or dry roasting peanuts and oilseed meals. Conway et al. (1978) evaluated the effect of roasting on aflatoxin-contaminated corn. Two commercial corn roasters were used; one was an electrically heated continuous cooker with the capacity of 150 lb/hr and the second was a gas-fired and continuous cooker with a capacity of 5000-6000 lbs of corn. Initial AFB_1 concentrations ranged from 133 to 877 ppb. Roasting reduced the concentration of AFB_1 by 40-80% after corn made a single passage through the continuous cooker. However, roasting did not reduce the level of AFB_1 below the FDA guidelines of 20 ppb (Conway et al., 1978). In a second study by Conway et al. (1978) the effect of roasting and ammonia to decontaminate corn containing aflatoxin was evaluated. Corn contaminated with AFB_1 ranging from 214 to 270 ppb was tempered to 20% moisture with aqueous ammonia and diluted to give 0.5% NH_3, then held for 3 hours before passing through a corn roaster. Using this procedure described above, aflatoxin was further reduced in excess of 90%. Of the five samples in the second portion of this experiment, only three were below the FDA guidelines of 20 ppb after treatment with ammonia and roasting (Conway et al., 1978). Temperature, heating interval and moisture content, can all affect the reduction of aflatoxin in corn,

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therefore it is difficult to achieve uniform destruction, making roasting a highly variable

method for detoxification. (Mann et al., 1967).

Solvent extraction

A number of solvents are capable of extracting aflatoxin from contaminated grains.

Advantages of this method include: aflatoxin can be completely removed under suitable

conditions, extraction can be carried out and solvent recovered with very little nutritional

loss. Limitations of this method include: special solvent extraction equipment is required,

added cost of processing and possible introduction of unpalatable flavors (McLean and

Dutton, 1995). Stahr and Obioha (1982) used methanolic extraction to decontaminate corn

containing 500 ppb of aflatoxin. Using one three-minute blending with methanol, 90% of the

aflatoxin was removed. The use of methanol for aflatoxin extraction is expensive; however,

recycling of methanol could be done by removal of aflatoxin by carbon or distillation of the

methanol (Stahr and Obioha, 1982).

Irradiation

Irradiation is the use of either non-ionizing radiation (NIR) or ionizing radiation (IR)

to inhibit aflatoxin formation during storage. IR involves X-rays, and gamma (γ) rays,

whereas, NIR involves UV rays, microwaves, infrared rays, and radio waves (Kabak et al., 2006). Aziz et al., (2002) found 3 kG γ irradiation to be effective at inhibiting A. *flavus* AFB_1 formation following 45 days of storage. However when only 2 kG γ irradiation was used, a small amount of AFB_1 (52.2ugkg-1) was produced, but it was much less than levels of AFB1 present in the non-irradiated control corn (Aziz et al., 2006). Additional studies conducted by Aziz and Smyk (2002) found that exposure to UV radiation induced the synthesis of AFB1 (200ppm) in non-toxigenic fungal strains of A. *flavus*. This indicates the inhibition of mold growth and mycotoxin biosynthesis is strain and dose dependent, as well as being influenced by humidity and storage conditions (Aziz et al., 2002; Zeinab et al., 2001).

Acid treatment

Treatment of aflatoxin-contaminated feed with a strong acid is thought to convert aflatoxin to its hemiacetal form through hydration. Pons et al. (1972) evaluated the effect of pH on the conversion of AFB_1 and AFG_1 to AFB_{2a} and AFG_{2a}, the latter compounds being much less toxic. It was found that as the pH decreased the rate of conversion increased. Although strong acids degraded AFB_1 and AFG_1, they had little effect on AFB_2 and AFG_2 (Pons et al., 1972). This study was conducted using aflatoxin-contaminated peanuts, however, the results may have implications for detoxifying contaminated corn.

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Alkaline treatment

Treatment with alkali, such as an organic or inorganic base has shown to be an effective and economically feasible method of degrading aflatoxin through hydrolysis of the lactone ring in AFB_1. Price et al. (1985), found that treatment of aflatoxin-contaminated corn with less than 0.5% of calcium hydroxide decreased aflatoxin levels by 43%. In 1989, Camou-Arriola and Price (1989) boiled corn naturally contaminated with 1600 ppb AFB_1 and AFB_2 with 3% NaOH at 100 C for 4 minutes, which resulted in a 93% decrease of AFB_1 and AFB_2 total levels. The decrease in aflatoxin-contaminated corn is directly related to the hydrolysis of the lactone ring, however evidence suggests that the hydrolyzed lactone ring can reform under acidic conditions and regenerate AFB_1 (Camou-Arriola and Price, 1989). Therefore treatment with an alkali may not be a reliable method for detoxification.

Sodium Bisulfite

Bisulfite is a reactive chemical that has the ability to: 1) inhibit growth of many microorganisms, 2) act as an antioxidant and 3) act as a reducing agent. It is thought that the reactive properties of sodium bisulfite can degrade aflatoxin, by reacting with the double bond at the 8,9 position of the terminal furan ring. Hagler et al. (1982) conducted a study to determine the detoxification potential of sodium bisulfite on AFB_1 contaminated corn. They

found AFB_1 was completely destroyed after soaking contaminated corn in 10% sodium bisulfite for 72 hours, followed by filtering, drying and incubation at 50 C for 21 days. The corn used in this study had aflatoxin levels in excess of 1900 ppb (Hagler et al., 1982). To determine if treatment with sodium bisulfite would be effective on corn with little AFB_1 contamination, Moerck et al. (1980) treated corn contaminated with levels of AFB_1 around 235 ppb with 0.5% and 1% sodium bisulfite. The results indicated using 0.5% and 1% were effective at destroying AFB_1 and more effective at destroying AFB_1 than NaOH or aqueous ammonia at the same level (Moerck et al., 1980). Detoxification of aflatoxin using sodium bisulfite has shown promising results however the addition of sodium bisulfate decreasing the palatability of the feed and therefore currently is not a practical strategy.

Ammoniation

Ammoniation uses gaseous ammonia or ammonium hydroxide to detoxify aflatoxin-contaminated corn. Ammoniation hydrolyzes the lactone ring, which allows chemical conversion of AFB_1 to less toxic products, such as aflatoxin D_1 (AFD_1). There are two ammoniation procedures currently used. One involves a high-pressure and high-temperature (HP/HT) process and the second uses atmospheric pressure and ambient temperature (AP/AT). The HP/HT method is primarily used to detoxify whole cottonseed, whereas the AP/AT method is used to detoxify corn. AP/AT is an approved detoxification method in

North Carolina and several other states (Cast, 2002). Brekke et al. (1977) published a study looking at inactivation of aflatoxin-contaminated corn using aqueous ammonia at atmospheric pressure. Ammonium hydroxide and tap water were added to aflatoxin-contaminated corn and mixed in a stainless steel drum for 30 minutes. The drum was held overnight at 25 C and then held for 12 days in a heated chamber to inactivate the aflatoxin. Ammoniation reduced total aflatoxin content from180 ug/kg to a non-detectable level in 12 days (Brekke et al., 1977). Brekke et al. (1978) conducted another study to evaluate aflatoxin inactivation using recycled ammonia (NH_3)-air mixture on whole and cracked corn). The NH_3-air mixture was recycled at 25 C through a glass column containing aflatoxin-contaminated corn. After the corn bed had reached a uniform distribution of NH_3 it was sealed and stored at 17.6% moisture and 25 C for 14 days. AFB_1 levels decreased from 1000ug/kg to 10ug/kg (Brekke et al., 1978). A down-side to using ammoniation as a detoxification method is the initial step is reversible, which may allow the lactone ring to reform if the reaction does not proceed to completion (Cast, 2002). In addition, the detoxified feed may have poor nutritional quality, off flavors, off odors, and toxic residues. Ammoniation is one of the most effective strategies for large quantity detoxification, due to the ability to decrease aflatoxin levels by more than 99%, however in the United States; the FDA does not allow ammoniation as a method of reducing aflatoxin levels in feed for interstate commerce (Brown 1999).

Ozonation

For many years ozone has been used for sanitation in the food processing industry.
More recently the ozonation process is being used to decontaminate grains containing
aflatoxin. This method produces ozone through the use of water and an electrolysis cell
(Mckenzie et al., 1998). Experiments conducted by Mckenzie et al. (1998) reduced aflatoxin
contamination in corn samples by 95% through the ozonation process. The ozonated corn
was then used in a second trial in which turkey poults were fed either aflatoxin-contaminated
corn or the ozone treated aflatoxin-contaminated corn. Turkeys fed the ozone treated corn did
not differ from controls in weight gain, liver weight, and blood chemistry; whereas those fed
non-treated aflatoxin-contaminated corn differed negatively when compared to the controls
(McKenzie et al., 1998). This study demonstrates that no harmful compounds were formed
through the ozonation process of detoxifying aflatoxin-contaminated corn. The studies
aforementioned indicate that ozonation may be a promising strategy for detoxifying
aflatoxin. Recent studies are in support of the previous results and find reduction of aflatoxin
in contaminated corn to be greater than 90% (Prudente and King, 2002). These studies have
also shown no reversion of inactivated aflatoxin to the toxic form (Prudente and King, 2002).
Ozonation has also proven effective in other feedstuffs such as cottonseed and peanut meals.
In a study conducted by Dwarakanath et al. (1968) treatment with ozone destroyed 91% of
AFB_1 in cottonseed meal after 2 hr and 78% of AFB_1 in peanut meal after 1 hr. For both

meals, AFB$_1$ was completely inactivated within the time specified (Dwarakanath et al., 1968). Since ozonation treatment is relatively new, further testing is needed to ensure safety, scalability and to determine any affects on nutritional characteristics of the feed.

Biological Inactivation

Early reports have indicated that aflatoxin may be susceptible to microbial modification. In 1966, Ciegler et al. (1966) evaluated yeasts, molds, bacteria, actinomycetes, algae and fungal spores for their ability to destroy or transform aflatoxin. In this experiment, AFB$_1$ was added to plates and flasks containing either yeasts, molds, bacteria, actinomycetes, algae or fungal spores. When AFB$_1$ was incubated with mold, some of the molds were able to partially transform AFB$_1$ to a new fluorescing compound. In particular, *Aspergillus niger* was able to occasionally degrade and modify aflatoxin; however incubation was required for a long period of time. When AFB$_1$ was incubated with *Flavobacterium aurantiacum* NRRL B-184, most of the aflatoxin was degraded. This led researchers to test the ability of *F. aurantiacum* to remove aflatoxin from solutions. Autoclaved *F. aurantiacum* was added to samples of milk, vegetable oil and peanut butter all contaminated with aflatoxin. When *F. aurantiacum* was incubated with milk containing 600 ug/50ml of AFB$_1$' complete removal of AFB$_1$ was observed after 2 hours. Similarly when *F. aurantiacum* was incubated with vegetable oil contaminated with 700 ug/50ml of AFB$_1$ and peanut butter contaminated with

700 ug/50 g of AFB_1, only trace amounts of AFB_1 were measured after 2 and 3 hours respectively. *Flavobacterium aurantiacum* was also incubated with 50 g of a variety of contaminated feedstuffs. It was found that corn contaminated with 800 ug of AFB_1 and peanuts contaminated with 648 ug of AFB_1 were 100% detoxified by the addition of *F. aurantiacum*. However, soybeans contaminated with 400 ug of AFB_1 were only 86% detoxified by the addition of *F. aurantiacum*. This study shows that microbial detoxification may be an effective strategy for reducing aflatoxin in feedstuffs. However, this study used feedstuffs artificially contaminated with aflatoxin, therefore more rigorous testing involving toxin removal from feedstuffs that are naturally contaminated with A. *flavus* is needed. Later studies conducted by Line and Brackett, (1995) showed that older culture (72 h) of *F. aurantiacum* were more effective at removing AFB_1 from solution then younger cultures (24-h or 48-h) as used in Ciegler et al. 1966. These same investigators also determined that certain acid-producing molds could catalyze hydration of aflatoxin B_1 to B_{2a}, which is a less-toxic product. The exact mechanism by which *F. aurantiacum* removes aflatoxin from food and feeds is still unknown, however a study by Lillehoj et al. (1967) found that AFB_1 interfered with *F. aurantiacum* cell wall synthesis and therefore concluded that AFB_1 was taken up by the cells, which caused the concentration of aflatoxin in the medium to decrease during the development of the organism. They also found that AFB_1 was not extractable after uptake only in living cells (Lillehoj et al., 1967). A study conducted by Line and Brackett. (1994) found that AFB_1 is degraded to water-soluble and chloroform-soluble products and

gaseous carbon dioxide (Line and Brackett, 1994). Current research examining the mechanism by which *F. aurantiacum* degrades AFB_1 suggests that a reductase system plays a role in the degradation of AFB1 by *F. aurantiacum* (D'souza and Brackett, 1998).

Hydrogen Peroxide

Research has been conducted on the use of hydrogen peroxide to detoxify food and feeds contaminated with mycotoxins (Sreenivasamurthy et al., 1967: Cater et al., 1974). Sreenivasamurthy et al. (1967) treated aqueous suspensions of peanut meal containing aflatoxin with 6% solutions of H_2O_2 and reported 97% of the toxin was destroyed and the treated meal that was fed to ducklings was not toxic.

Sequestering Agents

A final strategy to detoxify aflatoxin is through the use of sequestering agents. A sequestering agent is a substance that prevents or limits toxin absorption from the gastrointestinal tract of the animal. Ideally a sequestering should be effective against several mycotoxins, because feedstuffs are commonly contaminated with more than one mycotoxin. Types of sequestering agents include: activated carbons, cholestyramine, yeast cell wall derived products, and silicate minerals such as, phyllosilicates, zeolites, and hydrated sodium

calcium aluminosilicates. Effectiveness of different classes of sequestering agents to adsorb aflatoxin varies significantly. Bentonite, which is a phyllosilicate, has a layered crystalline microstructure that enables the adsorption of aflatoxin. Bentonites are comprised mainly of montmorillonite and have interchangeable cations. Many researchers have evaluated various types of bentonites for the ability to adsorb aflatoxin. An *in vitro* study conducted by Masimango et al. (1978) reported that a 2% inclusion rate of bentonite in a buffer solution resulted in the adsorption of 400 μg of AFB_1. Diaz et al. (2004) evaluated three sodium bentonites to reduce the transfer of AFB_1 to AFM_1. The three sodium bentonites (Astra Ben 20, Flow Guard, and Mycosorb) were included in the diet at 227 g/cow daily. The diet contained 100 ppb of aflatoxin. Astra Ben 20A®, Flow Guard® and Mycosorb® reduced the transfer of aflatoxin from feed to milk by 61, 65, and 50% respectively. Throughout the literature many of the bentonite products have consistently reduced the deleterious effects of aflatoxin (Lindemann et al. 1993, Stroud et al. 2006, and Wyatt 1991).

A group of sequestering agents that has not consistently shown to reduce the adsorption of aflatoxin is cell wall derived products. The cell wall derived products typically contain glucan polymers and/or mannan oligosaccharides. A study conducted by Diaz (2004) reported that MTB-100®, which is described as glucomannan containing yeast product, reduced milk aflatoxin in lactating Holstein cows by 59% when included in the diet at 10 g/cow daily. A few years later, Stroud et al. (2006) conducted a similar experiment and found

that MTB-100® did not reduce milk aflatoxin concentrations when fed to lactating Holstein cows at 100 g/cow daily. Previous inconsistent results regarding the efficacy of sequestering agents, has stimulated a great deal of research using these products.

AFLATOXIN SAMPLING

Detecting and quantifying the concentration of aflatoxin in foods and feeds destined for human and animal consumption is extremely important. The concentration of aflatoxin in the bulk lot is usually estimated by measuring the aflatoxin concentration in a small portion of the lot, or a sample taken from the lot. The aflatoxin concentration of a sample from the bulk lot is assumed to be the same concentration as the bulk lot. Based on the aflatoxin concentration of the bulk lot, the acceptability of the feedstuff for either human or animal consumption is compared to the legal limit. Common aflatoxin sampling techniques are usually a three step process. The steps are: sampling, sample preparation, and analysis. The sampling step outlines how the sample will be taken from the bulk lot and the sample size. The sample preparation step specifies the grinding of the sample to reduce particle size, and subsampling of the original ground sample. In the analytical step, aflatoxin is solvent-extracted from the ground subsample and quantified using approved procedures, such as high pressure liquid chromatography (HPLC), thin layer chromatography (TLC) or enzyme linked immunosorbent assay (ELISA).

There is significant variability associated with each of the above steps when determining aflatoxin concentration. Sampling is considered the step with greatest source of variability. In a study conducted by Whitaker et al. (1972), 6 lots of contaminated shelled peanuts were evaluated for aflatoxin. Each lot was analyzed for aflatoxin 10 times using TLC. All samples were ground and a 280 g subsample was removed. Among the replicated test results there was a large variability associated with estimating true aflatoxin concentration of the bulk lot. The standard deviations of the replicated samples within a lot ranged from 15 to 66 ppb. Studies conducted on a wide variety of agricultural products indicate that the sampling step, especially for small sample sizes, is usually the largest source of variability (Schatzki, 1995; Whitaker et al., 1976; Whitaker et al., 1993). Aflatoxin studies on corn and peanuts conducted by Whitaker et al. (1969) and Johansson et al. (2000) suggest that 0.1% of the kernels in a lot are contaminated and the concentration on a single kernel may be extremely high. Due to the extreme variability in aflatoxin concentrations in a lot Johansson et al. (2000), put forth an equation to estimate the sampling variance (VS) associated with testing aflatoxin concentrations in shelled corn. The equation states that for any sample size the sampling variance is a function of the lot concentration and the sample size : $VS=(12.95/ns)M^{0.98}$ where M is the aflatoxin concentration in the lot in ppb, and ns is the mass of shelled corn in the sample in kg.

In addition to sample size, another source of variability associated with sampling is the location from which the sample is taken. Harper et al. (2006) conducted an experiment to determine if depth within on-farm storage bin has an effect on aflatoxin concentration in corn contaminated with 57 ppb. In the experiment, four replicate samples (1100 g per sample) were collected from each of three depth regions in the bin, 1, 3, and 5 meters. Samples were ground and analyzed using ELISA. Aflatoxin concentration was significantly greater at 1-m sampling depth than at depths of 3 m and 5m. The authors suggested aflatoxin concentration was greater at 1 m depth because 1) aflatoxin production during storage occurred to a greater degree near the grain surface than in deeper regions of the bin and 2) the surface grain had a slightly greater moisture content which may have promoted aflatoxin production in this zone (Harper et al., 2006)

As previously mentioned, there is also variability in aflatoxin concentration due to sample preparation. Sample preparation is usually a two step process in which the sample is ground to reduce the particle size and a subsample is taken from the ground samples. As a result of this process there is variability among replicated subsamples, however the sample preparation variance is less than that of the variance associated with the sampling step due to the reduction of the subsample particle size. An equation to show the variance of sample preparation for aflatoxin in shelled corn is $VSS = (62.70/nss)M^{1.27}$, where M is aflatoxin concentration in the test sample in ppb, and nss is the mass of the shelled corn in the

subsample in grams . This equation reflects the use of a Romer mill to reduce particle size so that they will pass through a number 20 screen. The equation shows that the sample preparation variance is a function of the aflatoxin concentration in the sample and the size of the subsample.

Finally, there is variation among the analytical methods used to determine aflatoxin concentrations. Analytical methods involve several steps, such as solvent extraction, centrifugation, drying, dilution and quantification (Pohland and Trucksess, 2000). As a result there can be a disparity among replicated analyses on the same subsample. However, increasing the number of aliquots quantified by the chosen analytical method can reduce the variability associated with analytical testing (Whitaker, 2003).

Due to large variability from sampling, sample preparation and analytical testing, it is difficult to precisely determine the true aflatoxin concentration of a sample or the bulk lot, however implementing a mycotoxin sampling program can help to reduce error. The goal of any mycotoxin sampling program is reducing the variability in each procedure. The sampling variability can be reduced by increasing the size of the sample. Increasing the size of the subsample and/or increasing the number of particles per unit mass in the subsample will reduce the variability associated with sample preparation. The analytical variability can be reduced by either increasing the number of aliquots quantified or using a precise

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quantification method (Diaz, 2005). By reducing the variability of a mycotoxin test

procedure, the concentration of the contaminated bulk lot can more precisely be determined.

AFLATOXIN TESTING

Several methods are available for the detection and quantification of aflatoxin. The

most common methods used are High Pressure Liquid Chromatography (HPLC), Thin-Layer

Chromatography (TLC) and Enzyme linked Immunosorbent Assay (ELISA). HPLC is the

most frequently used method for mycotoxin analysis (Trucksess 1998). HPLC analysis is

very sensitive and can detect low levels of mycotoxins, in particular aflatoxin. HPLC

separates compounds present in an extract of a sample by relative affinity of the compounds

for a stationary column and mobile solvent. The eluted compounds pass through a detector

that helps quantitate the specific compounds in the original sample. Downsides to HPLC are

that extract from the sample usually requires substantial clean up before it can be injected

onto a column and the equipment required to run HPLC is expensive. However, HPLC tends

to be more precise than other methods currently available.

The ELISA method uses an antibody which distinguishes the three-dimensional structure of

mycotoxins. In general, ELISA is a high throughput assay with low sample volume

requirements and often has less sample clean up procedures compared to methods such as

TLC and HPLC. ELISA testing is simple, specific, and sensitive. It is quickly becoming the most common method for rapid detection of mycotoxins in food and feed (Zheng et al., 2005). One problem associated with ELISA testing is the fact that compounds with similar chemical groups can also interact with antibodies, because the target compounds are mycotoxins, not antigens. Therefore, ELISA methods can either overestimate or underestimate the mycotoxins in commodity samples (Zheng et al., 2005). A study conducted by Zheng et al. (2005) compared ELISA and HPLC methods to detect aflatoxins in grain and grain products. This study found that an ELISA microtiter plate for total aflatoxin was effectively comparable to HPLC for measuring total aflatoxin ranging from 4 to 40 ppb in corn and many other commodities. The ELISA method is also used to test for AFM_1 concentrations in milk. Abdel-Wahhab and Nada (1998) reported that for the detection of AFM_1, HPLC was the most accurate and detected AFM_1 concentrations as low as 0.125 ppb in milk samples. Samples analyzed using ELISA were accurate, however, the ELISA method was only able to detect AFM_1 concentrations greater than 0.25 ppb. A study conducted by Rosi et al. (2007) evaluated the reliability of ELISA to detect AFM1 in milk samples. Fifteen thousand samples were tested and the results from 600 were compared to results from HPLC. ELISA and HPLC assays gave the same precision, recovery and regression coefficient for samples containing less than 70 ng L-1 of AFM_1. However at higher concentrations of AFM_1, 100 ng L-1, ELISA gave a slight overestimation of AFM_1 compared to HPLC.

Although the literature reports HPLC to be more accurate overall, when considering time constraints and cost, ELISA may be a more favorable option.

Thin layer chromatography can be used as mycotoxin screening assay to determine the presence of one or more mycotoxins in a sample, however, TLC does not allow for quantitation (Trucksess 2001). Thin layer chromatography utilizes a glass plate on which a thin layer of silica gel has been placed. Extracts are spotted individually near one end of the plate along with standards. The end of plate with spotted extracts is placed in a specific solvent preparation, which covers the bottom of plate. The solvent is then adsorbed by the silica and travels up the plate through the standards and the spotted extracts. During this process various compounds in the extract spot are separated depending on their adsorption to the silica and their solubility. The compounds are deposited at different heights on the plate, indicating various mycotoxins (Cast, 2002).

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INTREPRETATIVE SUMMARY

Efficacy of Feed Additives to Bind Aflatoxin. *By Waltman et al.* Aflatoxins are a group of mycotoxins that routinely occur in feedstuffs, such as corn, peanuts and cottonseed. When diets containing aflatoxin B_1 are fed to lactating animals, a metabolite, aflatoxin M_1 is secreted into milk. Certain feed additives offer the potential to reduce transfer of aflatoxin from feed to milk and to reduce milk aflatoxin residues. The objective of this study was to determine the effect of sequestering agents on the change in milk aflatoxin concentrations when lactating dairy cattle consume aflatoxin-contaminated diets.

RUNNING HEAD: EFFICACY OF FEED ADDITIVES TO BIND AFLATOXIN

Effect of Sequestering Agents on Aflatoxin in Milk of Dairy Cows Fed Aflatoxin-contaminated Diets.

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ABSTRACT

Three experiments (**EXP**) were conducted to determine the potential of experimental

sequestering agents, clays or non-digestible yeast oligosaccharides, to reduce milk aflatoxin

concentration in lactating Holstein cows consuming aflatoxin. All EXP included two periods

in a randomized block design. Cows were fed an aflatoxin-contaminated total mixed ration

(**TMR**) for both periods of all trials. During the first period, cows received no sequestering

agents in the TMR, but agents were included in the TMR for the second period. EXP 1 and 2

consisted of two 7 d periods with 12 cows per treatment. Milk aflatoxin (**AFM$_1$**)

concentrations were analyzed by HPLC for milk samples collected on d 5 to 7 and d 11 to 13.

Two treatments in EXP 1 were: 1) control (no sequestering agent n=12), and 2)

100g/cow/day Lallemand n=12. Four treatments in EXP 2 were: control (no sequestering

agent) n=12, 2) 10g/cow/day MTB-100® (2004) n=12 (Alltech, Inc., Nicholasville, KY), 3)

10g/cow/day MTB-100® 2006 n=12 (Alltech, Inc., Nicholasville, KY) n=12, and 4)

10g/day/cow Alltech experimental (Alltech, Inc., Nicholasville, KY) n=12. EXP 3 consisted

of two 8 d periods and included 14 cows. Milk samples from d 4 to 8 and d 11 to 16 were

analyzed for AFM$_1$ concentrations by ELISA. Three treatments in EXP 3 were: 1) control

(no sequestering agent) n=4, 2) MTB-100® 2006 (Alltech, Inc., Nicholasville, KY) n=5 and

3) Astra-Ben 20A® (**AB-20A®**) (Prince Agri Products, Inc., Quincy, IL) n=5. For all EXPs,

the percent differences in AFM_1 concentrations between periods 1 and 2 were calculated. All percent differences were normalized using a correction factor that converted values for controls to zero. In EXP 1, the addition of the Lallemand product resulted in a 5.2% increase in AFM_1 concentration. In EXP 2, MTB-100® (2004), MTB-100® (2006), and Alltech experimental product resulted in 8.0%, 6.2%, and 9.5% increases in AFM_1 concentrations respectively. In EXP 3, MTB-100® (2006) resulted in a 5.1% decrease in AFM_1 concentrations, and AB-20A® resulted in a 60.4% decrease in AFM_1 concentrations. In summary, the AB-20A® in EXP 3 reduced AFM_1 concentrations however, there were no significant changes in AFM_1 concentrations in response to sequestering agents other than AB-20A®.

Key Words: Aflatoxin, Milk, Binder

Abbreviation key: **EXP** = Experiment; **AFM_1** = Aflatoxin M_1; **TMR** = Total Mixed Ration; **AB-20A®** = Astra-Ben 20A®.

INTRODUCTION

Aflatoxins are a group of mycotoxins produced by Aspergillus *flavus* and Aspergillus *parasiticus*. Aflatoxins are carcinogens that routinely occur in feed grains. A. *flavus* commonly contaminates corn, peanuts and cottonseed. Aflatoxins are considered unavoidable contaminants, in which contamination can occur during all stages of growth, harvest, storage and feeding. Aflatoxin B_1 (**AFB_1**) is the most common metabolite A. *flavus* that contaminates feed. When diets containing AFB_1 are fed to lactating animals, a metabolite, aflatoxin M_1 (**AFM_1**) is secreted into milk (Van Egmond and Paulsch, 1986). The formation of AFM_1 is via hydroxylation of AFB_1. Milk aflatoxin is toxic and carcinogenic, however, the toxicity of AFM_1 is reported to be less than the toxicity of the parent compound (AFB_1). (Hsieh et al., 1984; Sinnhuber et al., 1974). The transfer of aflatoxin from feed to milk is of concern for two reasons: first, due to the consumption of milk and milk products by humans, especially infants and children. Secondly, milk containing concentrations of AFM_1 greater than 0.5 ppb is not permitted for human consumption as stated by the Food and Drug Administration (Code of Federal Regulations Part 109). Therefore, milk containing AFM_1 above the action level must be discarded, which can cause significant economic loss for the dairy producer. Certain feed additives offer the potential to reduce transfer of aflatoxin from feed to milk and to reduce milk aflatoxin residues.

Sequestering agents, which are defined as products capable of attaching other substances to their surface without any chemical action. Sequestering agents have been used to reduce the absorption of aflatoxin in the feed and the gastrointestinal tract of lactating dairy cattle. Therefore, products can also protect many animals from the toxic effects of AFB_1 (Ramos et al., 1996). There have been several studies evaluating the ability of sequestering agents, such as, clays, activated carbons, and yeast products, to bind aflatoxin. *In vitro* studies have shown many of these products to be effective for the adsorption of aflatoxin (Diaz et al., 2004, Maryamma et al., 1991, Phillips et al., 1988). However, the *in vitro* binding ability of the sequestering agents does not necessarily predict the *in vivo* binding of the sequestering agents (Dwyer et al., 1997; Diaz et al., 1994; Stroud et al., 2006).

The objective of this study was to determine the effect of sequestering agents on the change in milk aflatoxin concentrations when lactating dairy cattle consume aflatoxin-contaminated diets.

MATERIAL AND METHODS

Diet

Naturally contaminated aflatoxin corn was used as a source of aflatoxin for the total mixed ration (**TMR**). Corn aflatoxin concentration was determined by North Carolina Department of Agriculture and Consumer Services Forage Laboratory. The corn was found

to be contaminated with approximately 1000 ppb of aflatoxin. Contaminated corn was mixed with molasses and corn oil (to eliminate inhaling aflatoxin). Contaminated corn was then ground and mixed thoroughly to ensure uniform distribution of aflatoxin in the corn. The corn was then blended into a total mixed ration in a mixer wagon. Aflatoxin-contaminated corn was mixed with uncontaminated corn in a 1 to 10 dilution so that total aflatoxin level in the feed would be approximately 100 ppb. At each feeding, one of the experimental sequestering agents was initially blended with soybean meal and then mixed with the appropriate TMR for each feeding group. Each treatment diet was blended using a DataRanger (American Calan Inc., Northwood, NH) mixer and fed to cows through Calan (American Calan Inc., Northwood, NH) feeding stations. After each treatment was fed, the mixer was flushed with 28 kg of silage to avoid cross contamination of sequestering agents.

Experimental Design

For all trials, lactating Holstein cows from the Piedmont Research Station in Salisbury, NC were randomly assigned to treatment groups. In experiment 1, 24 cows were assigned randomly to the following treatments: 1) control (no sequestering agent n=12), and 2) 100g/cow/day Lallemand n=12. Cows were in later lactation and produced 21.0 kg of milk/day. Diet dry matter (**DM**) contained 91.0 ppb of AFB_1. For experiment 2, 48 cows were assigned randomly among each of the following treatment groups: 1) control (no sequestering agent) n=12, 2) 10g/cow/day MTB-100[®] (2004) n=12 (Alltech, Inc.,

Nicholasville, KY), 3) 10g/cow/day MTB-100[®] (2006) n=12 (Alltech, Inc., Nicholasville,

KY) n=12, and 4) 10g/day/cow Alltech experimental (Alltech, Inc., Nicholasville, KY)

n=12. Cows were in later lactation and produced 21.2 kg of milk/day. Diet DM contained

94.0 ppb of AFB_1. For experiment 3, 14 cows were assigned to the following treatments 1)

control (no sequestering agent) n=4, 2) 10g/cow/daily MTB-100[®] (2006) (Alltech, Inc.,

Nicholasville, KY) n=5 and 3) 227g/cow/daily Astra-Ben 20A[®] (**AB-20A[®]**) (Prince Agri

Products, Inc., Quincy, IL) n=5. Cows were in later lactation and produced 19.7 kg of

milk/day. Diet DM contained 86.0 ppb of AFB_1. Experiments 1, 2 and 3 were designed as a

randomized block and blocked by milk production and parity. All experiments included two

periods, in which cows were fed an aflatoxin-contaminated TMR for both periods. During

the first period, cows received no sequestering agents in the TMR, but sequestering agents

were included in the TMR for the second period. For both periods, composite daily milk

samples of the total production were collected during the evening (1400h) and following

morning (0200) milkings. Experiment 1 and 2 consisted of two 7 day periods where milk

samples were collected on day 5 to 7, and 11 to 13 of the trial. In experiment 3, periods lasted

8 days and milk samples were collected on days 4 to 8 and d 11 to 16. Following the

conclusion of the collection periods of the experiment, milk samples were collected daily

until AFM_1 was cleared from the milk. Feed samples were collected daily to determine dry

matter. Diets were formulated to meet the nutrient requirements for the group average milk

production (NRC, 2001). To ensure total intake of the sequestering agent, feed consumption

was measured and then diets were allocated to minimize orts, resulting in a dry matter intake (**DMI**) of approximately 26 kg daily. All trials were approved by the Institutional Animal Care and Use Committee.

Aflatoxin M$_1$ Analysis

For all experiments milk samples were composited by day and milk production. A portion of milk from the evening sampling was combined with a portion of milk from the following morning sampling. The amount of milk composited from each sampling was determined based on the individual cow's milk yield for the two milking times. For experiment 1 and 2, milk samples were analyzed for AFM$_1$ using HPLC by Trilogy Analytical Laboratory, Washington, MO. For experiment 3, milk samples were analyzed for AFM$_1$ using the Ridascreen® Fast Aflatoxin M$_1$ ELISA. (R-Biopharm AG, Darmstadt, Germany).

Statistical Analysis

The experimental design was a complete randomized block design with cows blocked by milk production and parity. The efficacy of each treatment was evaluated based on the reduction in AFM$_1$ concentrations. For all experiments, statistical analyses were conducted using the GLM procedure of SAS (SAS®, 2001). The GLM procedure was used because there were not repeated measures. The model included the effects of treatment and error

$F_{(X)}$= treatment + error). The effects of the sequestering agents on AFM_1 were calculated as the percent difference in AFM_1 concentrations between period 1 and period 2, normalized to the control (the control was defined as having zero change). The changes in AFM_1 concentrations due to the sequestering agents were considered significant at $P < 0.05$. The main factors of the model used were treatment and cow. The effect of aflatoxin on DMI and milk yield was analyzed using the MIXED procedure of SAS (SAS®, 2001). The DMI for period 1, when cows were fed AFB_1 was covariately adjusted using pretreatment period, which was when cows received no aflatoxin-contaminated corn for 2 days prior to the experiment. The effect of aflatoxin on milk yield was analyzed using the same statistical model as used with DMI. Similarly, the effect of the sequestering agent on DMI and on milk yield was analyzed using the MIXED procedure of SAS (SAS®, 2001). The DMI for period 2, when cows were fed AFB_1 and a sequestering agent, was covariately adjusted using period 1, when cows were receiving only aflatoxin-contaminated corn. Again the same statistical model was used to determine the effect of the sequestering agent on milk yield.

RESULTS AND DISCUSSION

Of the sequestering agents used in the three trials, only AB-20A [®] resulted in a significant decrease in AFM_1 concentrations. In experiment 1, the Lallemand product resulted in a 5.2% increase in AFM_1 concentration. In experiment 2, MTB-100[®] (2004), MTB-100[®] (2006), and Alltech experimental product resulted in 8.0%, 6.2%, and 9.5%

increases in AFM_1 concentrations respectively. In experiment 3, MTB-100[®] (2006) resulted in a 5.1% decrease in AFM_1 concentrations, and AB-20A® resulted in a 60.4% decrease in AFM_1 concentrations (TABLE 1).

For experiments 1, 2 and 3, there was no effect of the sequestering agents on milk yield compared to controls (TABLE 2). Also, there was no effect of the sequestering agents on dry matter intake compared to controls (Table 3).

The ability of the sequestering agents to bind AFB_1 has been evaluated numerous times *in vitro* and *in vivo*. Diaz et al. (2004) reported that MTB-100[®] bound 96.6% of AFB_1 *in vitro* and reduced AFM_1 concentrations by 59% *in vivo* when fed to lactating Holstein cows at 10g/cow daily. Diaz et al. (2004) also reported that AB-20A[®] bound 98% of AFB_1 *in vitro*, however *in vivo* milk AFM_1 concentrations were reduced by 61% when lactating Holstein cows were fed AB-20A[®] at 227 g/cow daily. Stroud et al. (2006) also conducted *in vitro* and *in vivo* studies using both MTB-100[®] and AB-20A[®]. These *in vitro* studied reported that MTB-100[®] and AB-20A[®] bound 96.2% and 43.4% of AFB_1 respectively. A subsequent *in vivo* study conducted by Stroud et al. (2006) reported that MTB-100[®] did not reduce AFM_1 concentrations, however AB-20A[®] reduced AFM_1 concentrations in milk by 48.9% when lactating Holstein cows were fed 100g/cow daily of either AB-20A[®] or MTB-100[®]. Dwyer et al. (1997) reported a lack of predictability between *in vitro* adsorption data

and *in vivo* results. An experiment conducted by Kutz et al. (2008) reported that MTB-100® fed at 0.5% to lactating dairy cows did not significantly reduce milk aflatoxin concentrations.

Several studies in swine and poultry have found glucomannan containing yeast products to be effective at preventing the negative effects associated with feeding aflatoxin. An experiment conducted using poultry showed that yeast culture residue enhanced the performance of broiler breeder hens fed aflatoxin-contaminated diets (Stanley et al., 2004). Basmacioglu et al. (2005) reported the addition of an esterified glucan polymer, extracted from yeast cell wall reduced the deleterious effects caused by aflatoxin in broiler chickens (Basmacioglu et al., 2005).

The results from our trials confirm the work of Diaz et al. (2004) and Stroud et al. (2006) and show that AB-20A® can be used to decrease AFM_1 concentrations in milk from lactating Holstein cows. The results from this trial for MTB-100® are consistent with that of Stroud et al. (2006) and Kutz et al (2008), but in contrast to that of Diaz et al. (2004). This data suggests under the conditions of these trials, MTB-100® is not effective at reducing AFM_1 concentrations.

The efficacy of the Lallemand product to reduce AFM_1 concentrations has previously not been reported, however studies using sequestering agents comprised of montmorrilonite have shown montmorrilonite to effectively reduce the toxic effects of AFB_1 (Shi et al., 2007; Desheng et al., 2005). The results from this trial are not in support of Shi et al. (2007) and Desheng et al. (2005). However, this study was conducted in lactating Holstein cows,

whereas Shi et al. (2007) was conducted in swine and Desheng et al. (2005) was conducted in poultry. The exact composition of the Lallemand product is unknown, however it is referred to as a mixture of montmorillonite-diatomite and mannan-oligosaccharides (MOS), derived from yeast cell walls.

While yeast containing products have been shown to effectively reduce the toxic effects of aflatoxin in poultry and swine, research with ruminants is limited.

CONCLUSIONS

Bentonite (AB-20A[®]) fed at 227 g/cow daily reduced AFB_1 concentration by 60.4% when cows were fed diets containing 86.0 ppb of AFB_1. Experimental sequestering agents consisting of non-digestible yeast oligosaccharides (NYO) or mixtures of NYO and diatomite-montmorillonite used in this study did not affect AFM_1 concentrations when cows were fed diets containing 80 to 100 ppb AF.

TABLE 1. Percent reductions in milk aflatoxin concentration due to the addition of sequestering agents of cows fed 80 to 100 ppb aflatoxin-contaminated diet.

Sequestering Agents	Change in Milk Aflatoxin Concentration[1] (%)
Experiment 1	
Lallemand, 100g/d	+5.2
Experiment 2	
MTB-100® (2004), 10g/d	+8.0
MTB-100® (2006), 10g/d	+6.2
Alltech experimental, 10g/d	+9.5
Experiment 3	
MTB-100® (2006), 50g/d	-5.1
Astra Ben 20A®, 227g/d	-60.4*

[1] Not significantly different from control
*Values are different from control ($P < 0.05$)

TABLE 2. The effect of sequestering agents on milk yield of cows fed 80 to 100 ppb aflatoxin-contaminated diet.

Sequestering Agent	Average Daily Milk Yield (kg) [1]
Experiment 1	
Control (no sequestering agent)	20.4
Lallemand	20.0
Experiment 2	
Control	20.4
MTB-100[®] (2004)	19.8
MTB-100[®] (2006)	20.3
Alltech Experimental	20.4
Experiment 3	
Control	18
MTB-100[®] (2006)	20.5
Astra Ben 20A[®]	19.4
[1] Not significantly different from control	

TABLE 3. The effect of sequestering agents on daily dry matter intake of cows fed 80 to 100 ppb aflatoxin-contaminated diet.

Sequestering Agent	Average Daily Dry Matter Intake (kg) [1]
Experiment 1	
Control (no sequestering agent)	25.3
Lallemand	24.5
Experiment 2	
Control	25.3
MTB-100[®] (2004)	24.3
MTB-100[®] (2006)	23.0
Alltech Experimental	23.5
Experiment 3	
Control	23.3
MTB-100[®] (2006)	23.5
Astra Ben 20A[®]	23.5
[1] Not significantly different from control	

TABLE 4. Chemical analysis of ration fed to all cows in experiment 1, 2 and 3.

DM%	93.9
CP, % DM	16.3
TDN, % DM	69.9
ADF, % DM	23.9
Ca, % DM	0.46
P, % DM	0.50
Mg, % DM	0.18
Fe, ppm	160

TABLE 5. Ingredient composition of total mixed ration (% of dry matter) fed to all cows in experiment 1, 2, and 3.

Ingredient	% of Dry Matter
Corn Silage	64
Corn Grain	5.8
Aflatoxin Corn Grain	7.3
48 % Soybean Meal	10.5
Cottonseed Hulls	3.7
Whole Cottonseed	6.1
Lime	0.58
Dicalcium Phosphate	0.36
Salt	0.22
Bicarbonate	0.40
Trace Mineral-Vitamin	0.06
Dynamate	0.10

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APPENDICES

Ridascreen®Fast Aflatoxin M₁ Procedure

(R-Biopharm AG, Darmstadt, Germany)

The Ridascreen®Fast Aflatoxin M_1 test is based on an antigen-antibody reaction. Microtiter wells are coated with capture antibodies directed against ant-aflatoxin M_1 antibodies. Free aflatoxin M_1 and aflatoxin M_1 enzyme conjugate compete for the aflatoxin M_1 binding sites (competitive enzyme immunoassay). At the same time, anti-aflatoxin M_1 antibodies are bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Next substrate/chromogen is added to the wells and bound enzyme conjugate converts the chromogen into a blue product. Lastly, a stop solution is added and leads to a color change from blue to yellow. The measurement is made photometrically at 450nm. The absorbance is inversely proportional to the aflatoxin M_1 concentration in the sample.

1. Bring all reagents to room temperature
2. Insert wells into the microwell holder for all standards and samples that will be run
3. Pipet 50 µl of standard or prepared sample into separate wells
4. Add 50 µl of enzyme conjugate to each well

5. Add 50 µl of anti-aflatoxin M₁ antibody solution to each well. Mix gently by shaking the plate manually and incubate for 10 minutes at room temperature

6. Dump the liquid out of the wells. Tap the microwell holder upside down onto a clean filter towel (3 times in a row) to remove all remaining liquid from the wells.

7. Use a wash bottle or multichannel pipet to fill the wells with 250 µl of distilled water

8. Empty wells again and remove all remaining liquid (repeat the washing step 2 more times)

9. Add 100 µl of substrate/chromogen to each well.

10. Mix gently by shaking plate manually and incubate for 5 minutes at room temperature in the dark

11. Add 100 µl of the stop solution to each well

12. Mix gently by shaking the plate manually and measure the absorbance at 450 nm.

13. Read within 10 minutes of adding the stop solution.

High Performance Liquid Chromatography Procedure

(AOAC 2000.08)

The test portion is extracted and cleaned up by passing through an immunoaffinity column containing specific antibodies bound onto a solid support. Antibodies selectively bind with any aflatoxin M1(antigen) contained in the extract, to give an antibody–antigen complex. Other components of matrix are washed off the column with water. Aflatoxin M1 from the column is eluted with acetonitrile. After the eluate is concentrated, the amount of aflatoxin M1 is determined by LC with fluorometric detection.

1. Warm milk samples to 37 C in a water bath and then gently stir with a magnetic stirrer to disperse the fat layer

2. Centrifuge liquid milk at 2000 x g to separate the fat and discard the thin upper fat layer

3. Filter through filter paper, collecting at least 50 mL

4. Allow the immunoaffinity column to reach room temperature

5. Transfer 50 mL of prepared test portion with volumetric pipet into the syringe barrel and allow it to pass through the immunoaffinity column at a slow steady rate of ca 2-3 mL/min

6. Wash column with 20 mL of water at a steady flow rate

7. After washing completely, blow column dry with nitrogen steam

8. Put a clean dry barrel on the cartride

9. Slowly elute aflatoxin M_1 from the column with 4 mL of pure acetonitrile

10. Keep the acetonitrile in contact with the column for at least 60 seconds

11. Collect eluate in a conical tube

12. Evaporate eluate to dryness using a gentle stream of nitrogen

13. Dilute to volume V_f of mobile phase 200 μL (for 50 μL injections) to 1000 μL (for 250 μL injections)

14. Pump mobile phase at a steady flow rate through the LC column

15. Repeatedly inject a fixed amount of aflatoxin M_1 calibrant solution until stable peak areas or heights are obtained (Peak areas or heights corresponding to consecutive injections must be within ±5%)

16. Inject in sequence suitable volumes V_i of aflatoxin M_1 standard solutions containing from 0.05 to 1 ng

17. Prepare calibration graph by plotting the peak areas or peak height against the mass of the injected aflatoxin M_1

18. Inject a suitable volume V_i (equivalent to at least 12.5 mL of milk) of eluate onto the LC apparatus through the injection loop

19. Inject aflatoxin M_1 calibrant with every 10 injections

20. Determine the aflatoxin M_1 peak area or height corresponding to the analyte, and calculate the aflatoxin M_1 amount in the test material from the calibration graph, in ng

21. Calculate the aflatoxin M_1 mass concentration of the test sample using the following equation:

$$W_m = W_a \times (V_f / V_i) \times (1 / V_s)$$

Where W_m = the numerical value of aflatoxin M_1 in the test sample in ng/mL; W_s = the numerical value of aflatoxin M_1 corresponding to the area or height of the aflatoxin M_1 peak of the test extract (ng); V_f = the numerical value of the final volume of redissolved eluate (μL); V_i = the numerical value of the volume of injected eluate (μL); V_s = the numerical value of the volume of prepared test portion passing through the column (mL).

TABLE 6. Experiment 1, aflatoxin M_1 (ppb) concentration of milk from cows consuming approximately 80 to 100 ppb aflatoxin-contaminated diets without an sequestering agent (days 5 to 7) and with the inclusion of a sequestering agent (days 11 to 13), except for control that received no sequestering agent.

Cow No.	Treatment	Day in Trial	AFM_1 (ppb)
1675	control	5	1.37
1797	control	5	1.56
1855	control	5	1.85
1898	control	5	2.08
1924	control	5	1.32
1931	control	5	1.79
1970	control	5	2.12
1973	control	5	2.87
1978	control	5	3.11
3041	control	5	1.51
3047	control	5	4.05
3065	control	5	2.32
1745	Lallemand	5	1.02
1763	Lallemand	5	1.07
1865	Lallemand	5	2.18
1905	Lallemand	5	2.84
1934	Lallemand	5	2.17
1936	Lallemand	5	4.68
1957	Lallemand	5	1.83
1963	Lallemand	5	2.85
1966	Lallemand	5	3.99
3023	Lallemand	5	1.75
3035	Lallemand	5	2.45
3063	Lallemand	5	2.41
1675	control	6	2.70
1797	control	6	2.77
1855	control	6	1.75
1898	control	6	2.83
1924	control	6	2.93
1931	control	6	2.06
1970	control	6	2.37
1973	control	6	1.52

TABLE 6 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1978	control	6	0.60
3041	control	6	3.70
3047	control	6	3.71
3065	control	6	2.22
1745	Lallemand	6	2.02
1763	Lallemand	6	1.74
1865	Lallemand	6	3.42
1905	Lallemand	6	3.30
1934	Lallemand	6	2.27
1936	Lallemand	6	6.23
1957	Lallemand	6	2.00
1963	Lallemand	6	3.77
1966	Lallemand	6	1.90
3023	Lallemand	6	1.91
3035	Lallemand	6	1.96
3063	Lallemand	6	1.97
1675	control	7	1.86
1797	control	7	2.28
1855	control	7	1.28
1898	control	7	1.99
1924	control	7	1.72
1931	control	7	1.50
1970	control	7	1.73
1973	control	7	1.69
1978	control	7	0.77
3041	control	7	1.19
3047	control	7	2.60
3065	control	7	1.31
1745	Lallemand	7	1.57
1763	Lallemand	7	0.85
1865	Lallemand	7	2.51
1905	Lallemand	7	4.01
1934	Lallemand	7	2.43
1936	Lallemand	7	3.68
1957	Lallemand	7	1.21
1963	Lallemand	7	3.30

TABLE 6 (continued).

Cow No.	Treatment	Day in Trial	AFM_1 (ppb)
1966	Lallemand	7	2.40
3023	Lallemand	7	1.71
3035	Lallemand	7	1.40
3063	Lallemand	7	1.05
1675	control	11	1.69
1797	control	11	1.39
1855	control	11	1.28
1898	control	11	2.83
1924	control	11	0.97
1931	control	11	2.48
1970	control	11	2.10
1973	control	11	2.44
1978	control	11	1.58
3041	control	11	1.85
3047	control	11	3.75
3065	control	11	1.91
1745	Lallemand	11	1.52
1763	Lallemand	11	1.31
1865	Lallemand	11	1.29
1905	Lallemand	11	2.73
1934	Lallemand	11	1.69
1936	Lallemand	11	3.94
1957	Lallemand	11	1.56
1963	Lallemand	11	3.46
1966	Lallemand	11	2.36
3023	Lallemand	11	2.33
3035	Lallemand	11	2.10
3063	Lallemand	11	2.19
1675	control	12	1.20
1797	control	12	0.81
1855	control	12	1.31
1898	control	12	1.01
1924	control	12	2.63
1931	control	12	3.06
1970	control	12	1.35
1973	control	12	2.71

TABLE 6 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1978	control	12	1.74
3041	control	12	1.23
3047	control	12	3.27
3065	control	12	2.17
1745	Lallemand	12	1.54
1763	Lallemand	12	1.72
1865	Lallemand	12	1.85
1905	Lallemand	12	2.41
1934	Lallemand	12	2.16
1936	Lallemand	12	4.00
1957	Lallemand	12	2.04
1963	Lallemand	12	3.46
1966	Lallemand	12	3.13
3023	Lallemand	12	1.49
3035	Lallemand	12	1.79
3063	Lallemand	12	2.64
1675	control	13	1.10
1797	control	13	1.17
1855	control	13	1.35
1898	control	13	2.56
1924	control	13	2.60
1931	control	13	1.45
1970	control	13	1.13
1973	control	13	1.78
1978	control	13	1.14
3041	control	13	1.22
3047	control	13	2.16
3065	control	13	2.74
1745	Lallemand	13	1.20
1763	Lallemand	13	1.30
1865	Lallemand	13	1.59
1905	Lallemand	13	2.60
1934	Lallemand	13	2.07
1936	Lallemand	13	4.42
1957	Lallemand	13	1.95

TABLE 6 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1963	Lallemand	13	3.59
1966	Lallemand	13	2.29
3023	Lallemand	13	1.51
3035	Lallemand	13	1.66
3063	Lallemand	13	2.00

TABLE 7. Experiment 2, aflatoxin M$_1$ (ppb) concentration of milk from cows consuming approximately 80 to 100 ppb aflatoxin-contaminated diets without a sequestering agent (days 5 to 7) and with the inclusion of a sequestering agent (days 11 to 13), except for control that received no sequestering agent.

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1675	control	5	1.37
1797	control	5	1.56
1855	control	5	1.85
1898	control	5	2.08
1924	control	5	1.32
1931	control	5	1.79
1970	control	5	2.12
1973	control	5	2.87
1978	control	5	3.11
3041	control	5	1.51
3047	control	5	4.05
3065	control	5	2.32
1544	MTB-100 (2004)	5	1.38
1786	MTB-100 (2004)	5	0.99
1823	MTB-100 (2004)	5	4.46
1880	MTB-100 (2004)	5	1.76
1910	MTB-100 (2004)	5	0.87
1925	MTB-100 (2004)	5	3.21
1951	MTB-100 (2004)	5	1.93

TABLE 7 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1952	MTB-100 (2004)	5	3.35
1972	MTB-100 (2004)	5	1.38
1980	MTB-100 (2004)	5	1.88
1988	MTB-100 (2004)	5	4.47
3060	MTB-100 (2004)	5	1.83
1914	MTB-100 (2006)	5	1.37
1919	MTB-100 (2006)	5	2.05
1960	MTB-100 (2006)	5	2.86
1982	MTB-100 (2006)	5	2.21
1983	MTB-100 (2006)	5	2.60
2963	MTB-100 (2006)	5	2.38
3002	MTB-100 (2006)	5	4.61
3004	MTB-100 (2006)	5	2.52
3031	MTB-100 (2006)	5	5.82
3033	MTB-100 (2006)	5	5.02
3068	MTB-100 (2006)	5	2.33
3069	MTB-100 (2006)	5	1.78
1881	Alltech Experimental	5	1.35
1890	Alltech Experimental	5	2.39
1904	Alltech Experimental	5	2.33
1961	Alltech Experimental	5	2.05
2992	Alltech Experimental	5	1.78
3022	Alltech Experimental	5	4.10
3026	Alltech Experimental	5	2.99
3043	Alltech Experimental	5	3.41
3045	Alltech Experimental	5	3.57
3046	Alltech Experimental	5	8.12
3061	Alltech Experimental	5	1.88
3067	Alltech Experimental	5	2.11
1675	control	6	2.70
1797	control	6	2.77
1855	control	6	1.75
1898	control	6	2.83
1924	control	6	2.93
1931	control	6	2.06
1970	control	6	2.37

TABLE 7 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1973	control	6	1.52
1978	control	6	0.60
3041	control	6	3.70
3047	control	6	3.71
3065	control	6	2.22
1544	MTB-100 (2004)	6	1.73
1786	MTB-100 (2004)	6	1.45
1823	MTB-100 (2004)	6	3.92
1880	MTB-100 (2004)	6	1.70
1910	MTB-100 (2004)	6	2.37
1925	MTB-100 (2004)	6	3.57
1951	MTB-100 (2004)	6	2.58
1952	MTB-100 (2004)	6	2.54
1972	MTB-100 (2004)	6	1.41
1980	MTB-100 (2004)	6	2.46
1988	MTB-100 (2004)	6	3.02
3060	MTB-100 (2004)	6	2.05
1914	MTB-100 (2006)	6	2.46
1919	MTB-100 (2006)	6	1.84
1960	MTB-100 (2006)	6	2.03
1982	MTB-100 (2006)	6	1.53
1983	MTB-100 (2006)	6	1.83
2963	MTB-100 (2006)	6	2.17
3002	MTB-100 (2006)	6	4.04
3004	MTB-100 (2006)	6	2.20
3031	MTB-100 (2006)	6	6.27
3033	MTB-100 (2006)	6	5.04
3068	MTB-100 (2006)	6	2.92
3069	MTB-100 (2006)	6	2.37
1881	Alltech Experimental	6	1.08
1890	Alltech Experimental	6	2.73
1904	Alltech Experimental	6	2.83
1961	Alltech Experimental	6	1.78
2992	Alltech Experimental	6	2.42
3022	Alltech Experimental	6	4.62
3026	Alltech Experimental	6	2.79

TABLE 7 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
3043	Alltech Experimental	6	2.31
3045	Alltech Experimental	6	3.74
3046	Alltech Experimental	6	6.34
3061	Alltech Experimental	6	2.22
3067	Alltech Experimental	6	2.35
1675	control	7	1.86
1797	control	7	2.28
1855	control	7	1.28
1898	control	7	1.99
1924	control	7	1.72
1931	control	7	1.50
1970	control	7	1.73
1973	control	7	1.69
1978	control	7	0.77
3041	control	7	1.19
3047	control	7	2.60
3065	control	7	1.31
1544	MTB-100 (2004)	7	1.33
1786	MTB-100 (2004)	7	1.70
1823	MTB-100 (2004)	7	3.95
1880	MTB-100 (2004)	7	1.35
1910	MTB-100 (2004)	7	1.59
1925	MTB-100 (2004)	7	3.06
1951	MTB-100 (2004)	7	2.64
1952	MTB-100 (2004)	7	2.20
1972	MTB-100 (2004)	7	0.96
1980	MTB-100 (2004)	7	1.80
1988	MTB-100 (2004)	7	4.94
3060	MTB-100 (2004)	7	1.32
1914	MTB-100 (2006)	7	1.72
1919	MTB-100 (2006)	7	2.10
1960	MTB-100 (2006)	7	2.53
1982	MTB-100 (2006)	7	1.03
1983	MTB-100 (2006)	7	1.32
2963	MTB-100 (2006)	7	1.47
3002	MTB-100 (2006)	7	3.10

TABLE 7 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
3004	MTB-100 (2006)	7	1.56
3031	MTB-100 (2006)	7	4.36
3033	MTB-100 (2006)	7	3.01
3068	MTB-100 (2006)	7	1.21
3069	MTB-100 (2006)	7	2.26
1881	Alltech Experimental	7	1.12
1890	Alltech Experimental	7	2.81
1904	Alltech Experimental	7	2.36
1961	Alltech Experimental	7	2.08
2992	Alltech Experimental	7	1.38
3022	Alltech Experimental	7	5.17
3026	Alltech Experimental	7	2.92
3043	Alltech Experimental	7	1.19
3045	Alltech Experimental	7	2.45
3046	Alltech Experimental	7	3.64
3061	Alltech Experimental	7	2.29
3067	Alltech Experimental	7	1.51
1675	control	11	1.69
1797	control	11	1.39
1855	control	11	1.28
1898	control	11	2.83
1924	control	11	0.97
1931	control	11	2.48
1970	control	11	2.10
1973	control	11	2.44
1978	control	11	1.58
3041	control	11	1.85
3047	control	11	3.75
3065	control	11	1.91
1544	MTB-100 (2004)	11	1.67
1786	MTB-100 (2004)	11	1.53
1823	MTB-100 (2004)	11	3.05
1880	MTB-100 (2004)	11	2.25
1910	MTB-100 (2004)	11	1.72
1925	MTB-100 (2004)	11	2.78
1951	MTB-100 (2004)	11	3.11

TABLE 7 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1952	MTB-100 (2004)	11	2.01
1972	MTB-100 (2004)	11	1.54
1980	MTB-100 (2004)	11	1.14
1988	MTB-100 (2004)	11	1.74
3060	MTB-100 (2004)	11	2.44
1914	MTB-100 (2006)	11	1.71
1919	MTB-100 (2006)	11	1.75
1960	MTB-100 (2006)	11	2.63
1982	MTB-100 (2006)	11	1.94
1983	MTB-100 (2006)	11	2.94
2963	MTB-100 (2006)	11	1.48
3002	MTB-100 (2006)	11	1.82
3004	MTB-100 (2006)	11	1.70
3031	MTB-100 (2006)	11	5.00
3033	MTB-100 (2006)	11	3.39
3068	MTB-100 (2006)	11	2.84
3069	MTB-100 (2006)	11	1.85
1881	Alltech Experimental	11	1.27
1890	Alltech Experimental	11	2.00
1904	Alltech Experimental	11	3.88
1961	Alltech Experimental	11	2.09
2992	Alltech Experimental	11	1.70
3022	Alltech Experimental	11	5.29
3026	Alltech Experimental	11	1.73
3043	Alltech Experimental	11	2.09
3045	Alltech Experimental	11	2.55
3046	Alltech Experimental	11	4.86
3061	Alltech Experimental	11	3.12
3067	Alltech Experimental	11	1.60
1675	control	12	1.20
1797	control	12	0.81
1855	control	12	1.31
1898	control	12	1.01
1924	control	12	2.63
1931	control	12	3.06
1970	control	12	1.35

TABLE 7 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1973	control	12	2.71
1978	control	12	1.74
3041	control	12	1.23
3047	control	12	3.27
3065	control	12	2.17
1544	MTB-100 (2004)	12	1.53
1786	MTB-100 (2004)	12	1.27
1823	MTB-100 (2004)	12	2.80
1880	MTB-100 (2004)	12	1.37
1910	MTB-100 (2004)	12	1.93
1925	MTB-100 (2004)	12	3.51
1951	MTB-100 (2004)	12	3.57
1952	MTB-100 (2004)	12	2.82
1972	MTB-100 (2004)	12	2.18
1980	MTB-100 (2004)	12	1.53
1988	MTB-100 (2004)	12	3.24
3060	MTB-100 (2004)	12	1.93
1914	MTB-100 (2006)	12	1.92
1919	MTB-100 (2006)	12	2.28
1960	MTB-100 (2006)	12	2.57
1982	MTB-100 (2006)	12	1.37
1983	MTB-100 (2006)	12	2.24
2963	MTB-100 (2006)	12	2.64
3002	MTB-100 (2006)	12	2.65
3004	MTB-100 (2006)	12	1.55
3031	MTB-100 (2006)	12	3.73
3033	MTB-100 (2006)	12	3.93
3068	MTB-100 (2006)	12	1.71
3069	MTB-100 (2006)	12	2.31
1881	Alltech Experimental	12	1.50
1890	Alltech Experimental	12	1.68
1904	Alltech Experimental	12	3.02
1961	Alltech Experimental	12	2.65
2992	Alltech Experimental	12	1.99
3022	Alltech Experimental	12	4.61
3026	Alltech Experimental	12	2.58

TABLE 7 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
3043	Alltech Experimental	12	2.41
3045	Alltech Experimental	12	2.12
3046	Alltech Experimental	12	4.48
3061	Alltech Experimental	12	2.80
3067	Alltech Experimental	12	1.40
1675	control	13	1.10
1797	control	13	1.17
1855	control	13	1.35
1898	control	13	2.56
1924	control	13	2.60
1931	control	13	1.45
1970	control	13	1.13
1973	control	13	1.78
1978	control	13	1.14
3041	control	13	1.22
3047	control	13	2.16
3065	control	13	2.74
1544	MTB-100 (2004)	13	1.46
1786	MTB-100 (2004)	13	1.59
1823	MTB-100 (2004)	13	2.22
1880	MTB-100 (2004)	13	1.32
1910	MTB-100 (2004)	13	1.72
1925	MTB-100 (2004)	13	2.56
1951	MTB-100 (2004)	13	2.20
1952	MTB-100 (2004)	13	2.05
1972	MTB-100 (2004)	13	1.19
1980	MTB-100 (2004)	13	1.42
1988	MTB-100 (2004)	13	3.53
3060	MTB-100 (2004)	13	2.52
1914	MTB-100 (2006)	13	1.88
1919	MTB-100 (2006)	13	2.10
1960	MTB-100 (2006)	13	2.12
1982	MTB-100 (2006)	13	1.56
1983	MTB-100 (2006)	13	2.09
2963	MTB-100 (2006)	13	2.06
3002	MTB-100 (2006)	13	3.81

TABLE 7 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
3004	MTB-100 (2006)	13	2.12
3031	MTB-100 (2006)	13	2.56
3033	MTB-100 (2006)	13	5.55
3068	MTB-100 (2006)	13	2.62
3069	MTB-100 (2006)	13	1.87
1881	Alltech Experimental	13	1.80
1890	Alltech Experimental	13	1.81
1904	Alltech Experimental	13	2.87
1961	Alltech Experimental	13	2.44
2992	Alltech Experimental	13	1.63
3022	Alltech Experimental	13	4.73
3026	Alltech Experimental	13	1.09
3043	Alltech Experimental	13	1.63
3045	Alltech Experimental	13	4.44
3046	Alltech Experimental	13	6.53
3061	Alltech Experimental	13	2.16
3067	Alltech Experimental	13	2.39

TABLE 8. Experiment 3, aflatoxin M$_1$ (ppb) concentration of milk from cows consuming approximately 80 to 100 ppb aflatoxin-contaminated diets without a sequestering agent (days 4 to 8) and with the inclusion of a sequestering agent (days 11 to 15), except for control that received no sequestering agent.

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1833	Control	4	1.66
1904	Control	4	1.54
1983	Control	4	1.74
3041	Control	4	1.59
1747	MTB-100 (2006)	4	1.00
1762	MTB-100 (2006)	4	1.57
1828	MTB-100 (2006)	4	1.24
1891	MTB-100 (2006)	4	1.56
3043	MTB-100 (2006)	4	1.86

TABLE 8 (continued).

Cow No.	Treatment	Day in Trial	AFM_1 (ppb)
1753	AB-20A	4	1.45
1791	AB-20A	4	1.56
1803	AB-20A	4	1.43
1982	AB-20A	4	1.65
3032	AB-20A	4	1.47
1833	Control	5	1.74
1904	Control	5	1.79
1983	Control	5	1.96
3041	Control	5	1.44
1747	MTB-100 (2006)	5	1.45
1762	MTB-100 (2006)	5	1.75
1828	MTB-100 (2006)	5	1.41
1891	MTB-100 (2006)	5	1.76
3043	MTB-100 (2006)	5	1.55
1753	AB-20A	5	1.83
1791	AB-20A	5	1.36
1803	AB-20A	5	1.42
1982	AB-20A	5	1.24
3032	AB-20A	5	1.35
1833	Control	6	1.59
1904	Control	6	1.59
1983	Control	6	1.82
3041	Control	6	1.74
1747	MTB-100 (2006)	6	1.77
1762	MTB-100 (2006)	6	1.95
1828	MTB-100 (2006)	6	1.54
1891	MTB-100 (2006)	6	1.75
3043	MTB-100 (2006)	6	1.48
1753	AB-20A	6	1.43
1791	AB-20A	6	1.34
1803	AB-20A	6	1.71
1982	AB-20A	6	1.57
3032	AB-20A	6	1.73
1833	Control	7	1.72
1904	Control	7	1.55
1983	Control	7	1.51

TABLE 8 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
3041	Control	7	1.86
1747	MTB-100 (2006)	7	1.89
1762	MTB-100 (2006)	7	1.83
1828	MTB-100 (2006)	7	1.83
1891	MTB-100 (2006)	7	1.76
3043	MTB-100 (2006)	7	1.93
1753	AB-20A	7	1.71
1791	AB-20A	7	1.40
1803	AB-20A	7	1.50
1982	AB-20A	7	1.69
3032	AB-20A	7	1.54
1833	Control	8	1.90
1904	Control	8	1.80
1983	Control	8	1.72
3041	Control	8	1.41
1747	MTB-100 (2006)	8	1.60
1762	MTB-100 (2006)	8	1.59
1828	MTB-100 (2006)	8	1.50
1891	MTB-100 (2006)	8	1.70
3043	MTB-100 (2006)	8	1.52
1753	AB-20A	8	0.99
1791	AB-20A	8	0.86
1803	AB-20A	8	0.73
1982	AB-20A	8	0.81
3032	AB-20A	8	0.75
1833	Control	11	1.71
1904	Control	11	1.43
1983	Control	11	1.78
3041	Control	11	1.58
1747	MTB-100 (2006)	11	1.26
1762	MTB-100 (2006)	11	1.40
1828	MTB-100 (2006)	11	1.75
1891	MTB-100 (2006)	11	1.47
3043	MTB-100 (2006)	11	1.72
1753	AB-20A	11	0.66
1791	AB-20A	11	0.70

TABLE 8 (continued).

Cow No.	Treatment	Day in Trial	AFM$_1$ (ppb)
1803	AB-20A	11	0.69
1982	AB-20A	11	0.66
3032	AB-20A	11	0.55
1833	Control	12	1.61
1904	Control	12	1.95
1983	Control	12	1.51
3041	Control	12	1.64
1747	MTB-100 (2006)	12	1.53
1762	MTB-100 (2006)	12	1.47
1828	MTB-100 (2006)	12	1.24
1891	MTB-100 (2006)	12	1.33
3043	MTB-100 (2006)	12	1.64
1753	AB-20A	12	0.50
1791	AB-20A	12	0.45
1803	AB-20A	12	0.53
1982	AB-20A	12	0.57
3032	AB-20A	12	0.59
1833	Control	13	1.48
1904	Control	13	1.22
1983	Control	13	1.80
3041	Control	13	1.44
1747	MTB-100 (2006)	13	1.10
1762	MTB-100 (2006)	13	1.70
1828	MTB-100 (2006)	13	1.03
1891	MTB-100 (2006)	13	2.10
3043	MTB-100 (2006)	13	2.05
1753	AB-20A	13	0.52
1791	AB-20A	13	0.49
1803	AB-20A	13	0.61
1982	AB-20A	13	0.58
3032	AB-20A	13	0.51
1833	Control	14	1.84
1904	Control	14	1.73
1983	Control	14	1.30
3041	Control	14	1.90
1747	MTB-100 (2006)	14	1.97

TABLE 8 (continued).

Cow No.	Treatment	Day in Trial	AFM_1 (ppb)
1762	MTB-100 (2006)	14	1.27
1828	MTB-100 (2006)	14	1.48
1891	MTB-100 (2006)	14	1.77
3043	MTB-100 (2006)	14	1.35
1753	AB-20A	14	0.57
1791	AB-20A	14	0.61
1803	AB-20A	14	0.49
1982	AB-20A	14	0.48
3032	AB-20A	14	0.60
1833	Control	15	1.32
1904	Control	15	1.23
1983	Control	15	1.32
3041	Control	15	1.53
1747	MTB-100 (2006)	15	1.53
1762	MTB-100 (2006)	15	1.45
1828	MTB-100 (2006)	15	1.35
1891	MTB-100 (2006)	15	1.34
3043	MTB-100 (2006)	15	1.70
1753	AB-20A	15	0.62
1791	AB-20A	15	0.49
1803	AB-20A	15	0.53
1982	AB-20A	15	0.56
3032	AB-20A	15	0.52

TABLE 9. Experiment 1, milk yield (kg) from cows consuming approximately 80 to 100 ppb aflatoxin-contaminated diets without a sequestering agent (days 4 to 8) and with the inclusion of a sequestering agent (days 11 to 15), except for control that received no sequestering agent.

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1675	Control	5	16.45
1797	Control	5	8.41
1855	Control	5	23.06

TABLE 9 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1898	Control	5	19.64
1924	Control	5	16.14
1931	Control	5	25.77
1970	Control	5	15.45
1973	Control	5	19.59
1978	Control	5	27.97
3041	Control	5	29.20
3047	Control	5	23.76
3065	Control	5	21.24
1745	Lallemand	5	26.12
1763	Lallemand	5	21.34
1865	Lallemand	5	17.76
1905	Lallemand	5	25.76
1934	Lallemand	5	22.74
1936	Lallemand	5	14.65
1957	Lallemand	5	23.68
1963	Lallemand	5	18.32
1966	Lallemand	5	25.26
3023	Lallemand	5	19.71
3035	Lallemand	5	19.57
3063	Lallemand	5	19.14
1675	Control	6	16.50
1797	Control	6	.
1855	Control	6	23.14
1898	Control	6	20.95
1924	Control	6	13.09
1931	Control	6	.
1970	Control	6	12.14
1973	Control	6	19.95
1978	Control	6	28.50
3041	Control	6	28.82
3047	Control	6	23.45
3065	Control	6	20.68
1745	Lallemand	6	25.59
1763	Lallemand	6	.
1865	Lallemand	6	17.05

TABLE 9 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1905	Lallemand	6	24.91
1934	Lallemand	6	20.59
1936	Lallemand	6	14.50
1957	Lallemand	6	22.55
1963	Lallemand	6	17.50
1966	Lallemand	6	23.05
3023	Lallemand	6	18.73
3035	Lallemand	6	.
3063	Lallemand	6	18.68
1675	Control	7	14.50
1797	Control	7	9.32
1855	Control	7	22.41
1898	Control	7	19.18
1924	Control	7	16.95
1931	Control	7	26.95
1970	Control	7	18.14
1973	Control	7	19.45
1978	Control	7	28.73
3041	Control	7	30.91
3047	Control	7	23.91
3065	Control	7	21.36
1745	Lallemand	7	26.59
1763	Lallemand	7	22.23
1865	Lallemand	7	18.55
1905	Lallemand	7	25.86
1934	Lallemand	7	24.91
1936	Lallemand	7	15.27
1957	Lallemand	7	24.50
1963	Lallemand	7	18.77
1966	Lallemand	7	26.14
3023	Lallemand	7	18.73
3035	Lallemand	7	19.95
3063	Lallemand	7	19.00
1675	Control	11	10.82
1797	Control	11	6.55
1855	Control	11	24.14

TABLE 9 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1898	Control	11	18.09
1924	Control	11	21.91
1931	Control	11	23.91
1970	Control	11	18.32
1973	Control	11	19.86
1978	Control	11	26.23
3041	Control	11	28.95
3047	Control	11	20.09
3065	Control	11	20.14
1745	Lallemand	11	25.55
1763	Lallemand	11	19.18
1865	Lallemand	11	14.82
1905	Lallemand	11	23.23
1934	Lallemand	11	20.91
1936	Lallemand	11	11.95
1957	Lallemand	11	22.50
1963	Lallemand	11	17.64
1966	Lallemand	11	21.73
3023	Lallemand	11	18.00
3035	Lallemand	11	18.41
3063	Lallemand	11	19.00
1675	Control	12	16.32
1797	Control	12	6.95
1855	Control	12	20.91
1898	Control	12	17.91
1924	Control	12	23.32
1931	Control	12	26.77
1970	Control	12	13.09
1973	Control	12	19.50
1978	Control	12	26.45
3041	Control	12	26.55
3047	Control	12	21.55
3065	Control	12	21.18
1745	Lallemand	12	24.73
1763	Lallemand	12	21.50
1865	Lallemand	12	16.73

TABLE 9 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1905	Lallemand	12	24.64
1934	Lallemand	12	22.73
1936	Lallemand	12	14.18
1957	Lallemand	12	24.27
1963	Lallemand	12	18.86
1966	Lallemand	12	24.18
3023	Lallemand	12	20.50
3035	Lallemand	12	19.82
3063	Lallemand	12	16.14
1675	Control	13	12.18
1797	Control	13	7.18
1855	Control	13	22.82
1898	Control	13	17.36
1924	Control	13	22.50
1931	Control	13	25.09
1970	Control	13	18.59
1973	Control	13	19.05
1978	Control	13	27.82
3041	Control	13	28.50
3047	Control	13	23.09
3065	Control	13	21.09
1745	Lallemand	13	28.32
1763	Lallemand	13	22.41
1865	Lallemand	13	17.73
1905	Lallemand	13	26.05
1934	Lallemand	13	22.36
1936	Lallemand	13	13.91
1957	Lallemand	13	23.41
1963	Lallemand	13	17.59
1966	Lallemand	13	23.05
3023	Lallemand	13	20.00
3035	Lallemand	13	19.05
3063	Lallemand	13	16.73

TABLE 10. Experiment 2, milk yield (kg) from cows consuming approximately 80 to 100 ppb aflatoxin-contaminated diets without a sequestering agent (days 4 to 8) and with the inclusion of a sequestering agent (days 11 to 15), except for control that received no sequestering agent.

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1675	Control	5	16.45
1797	Control	5	8.41
1855	Control	5	23.06
1898	Control	5	19.64
1924	Control	5	16.14
1931	Control	5	25.77
1970	Control	5	15.45
1973	Control	5	19.59
1978	Control	5	27.97
3041	Control	5	29.20
3047	Control	5	23.76
3065	Control	5	21.24
1544	MTB-100 (2004)	5	24.23
1786	MTB-100 (2004)	5	13.20
1823	MTB-100 (2004)	5	11.41
1880	MTB-100 (2004)	5	20.79
1910	MTB-100 (2004)	5	15.76
1925	MTB-100 (2004)	5	20.05
1951	MTB-100 (2004)	5	26.20
1952	MTB-100 (2004)	5	25.83
1972	MTB-100 (2004)	5	19.39
1980	MTB-100 (2004)	5	17.82
1988	MTB-100 (2004)	5	16.80
3060	MTB-100 (2004)	5	23.35
1914	MTB-100 (2006)	5	20.27
1919	MTB-100 (2006)	5	15.32
1960	MTB-100 (2006)	5	19.45
1982	MTB-100 (2006)	5	28.09
1983	MTB-100 (2006)	5	27.59
2963	MTB-100 (2006)	5	23.77
3002	MTB-100 (2006)	5	15.09
3004	MTB-100 (2006)	5	13.05

TABLE 10 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
3031	MTB-100 (2006)	5	23.35
3033	MTB-100 (2006)	5	22.52
3068	MTB-100 (2006)	5	25.83
3069	MTB-100 (2006)	5	22.52
1881	Alltech Experimental	5	11.20
1890	Alltech Experimental	5	21.20
1904	Alltech Experimental	5	22.70
1961	Alltech Experimental	5	15.61
2992	Alltech Experimental	5	17.62
3022	Alltech Experimental	5	31.50
3026	Alltech Experimental	5	25.42
3043	Alltech Experimental	5	29.85
3045	Alltech Experimental	5	20.89
3046	Alltech Experimental	5	21.52
3061	Alltech Experimental	5	19.14
3067	Alltech Experimental	5	19.41
1675	Control	6	16.50
1797	Control	6	.
1855	Control	6	23.14
1898	Control	6	20.95
1924	Control	6	13.09
1931	Control	6	.
1970	Control	6	12.14
1973	Control	6	19.95
1978	Control	6	28.50
3041	Control	6	28.82
3047	Control	6	23.45
3065	Control	6	20.68
1544	MTB-100 (2004)	6	24.91
1786	MTB-100 (2004)	6	14.00
1823	MTB-100 (2004)	6	16.14
1880	MTB-100 (2004)	6	21.32
1910	MTB-100 (2004)	6	15.36
1925	MTB-100 (2004)	6	19.00
1951	MTB-100 (2004)	6	26.36
1952	MTB-100 (2004)	6	24.09

TABLE 10 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1972	MTB-100 (2004)	6	.
1980	MTB-100 (2004)	6	16.59
1988	MTB-100 (2004)	6	12.45
3060	MTB-100 (2004)	6	16.73
1914	MTB-100 (2006)	6	19.73
1919	MTB-100 (2006)	6	16.23
1960	MTB-100 (2006)	6	13.59
1982	MTB-100 (2006)	6	27.55
1983	MTB-100 (2006)	6	26.45
2963	MTB-100 (2006)	6	23.77
3002	MTB-100 (2006)	6	14.50
3004	MTB-100 (2006)	6	11.27
3031	MTB-100 (2006)	6	22.41
3033	MTB-100 (2006)	6	22.55
3068	MTB-100 (2006)	6	24.59
3069	MTB-100 (2006)	6	21.55
1881	Alltech Experimental	6	13.09
1890	Alltech Experimental	6	21.64
1904	Alltech Experimental	6	23.64
1961	Alltech Experimental	6	12.23
2992	Alltech Experimental	6	18.64
3022	Alltech Experimental	6	27.18
3026	Alltech Experimental	6	24.55
3043	Alltech Experimental	6	28.18
3045	Alltech Experimental	6	21.86
3046	Alltech Experimental	6	22.09
3061	Alltech Experimental	6	18.55
3067	Alltech Experimental	6	18.27
1675	Control	7	14.50
1797	Control	7	9.32
1855	Control	7	22.41
1898	Control	7	19.18
1924	Control	7	16.95
1931	Control	7	26.95
1970	Control	7	18.14
1973	Control	7	19.45

TABLE 10 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1978	Control	7	28.73
3041	Control	7	30.91
3047	Control	7	23.91
3065	Control	7	21.36
1544	MTB-100 (2004)	7	22.73
1786	MTB-100 (2004)	7	12.41
1823	MTB-100 (2004)	7	8.91
1880	MTB-100 (2004)	7	21.05
1910	MTB-100 (2004)	7	16.45
1925	MTB-100 (2004)	7	20.23
1951	MTB-100 (2004)	7	25.82
1952	MTB-100 (2004)	7	27.05
1972	MTB-100 (2004)	7	20.18
1980	MTB-100 (2004)	7	19.18
1988	MTB-100 (2004)	7	19.82
3060	MTB-100 (2004)	7	25.05
1914	MTB-100 (2006)	7	21.36
1919	MTB-100 (2006)	7	14.77
1960	MTB-100 (2006)	7	21.59
1982	MTB-100 (2006)	7	27.86
1983	MTB-100 (2006)	7	27.18
2963	MTB-100 (2006)	7	22.41
3002	MTB-100 (2006)	7	15.64
3004	MTB-100 (2006)	7	12.50
3031	MTB-100 (2006)	7	25.68
3033	MTB-100 (2006)	7	21.86
3068	MTB-100 (2006)	7	26.45
3069	MTB-100 (2006)	7	23.27
1881	Alltech Experimental	7	12.50
1890	Alltech Experimental	7	20.14
1904	Alltech Experimental	7	21.59
1961	Alltech Experimental	7	16.82
2992	Alltech Experimental	7	17.18
3022	Alltech Experimental	7	34.36
3026	Alltech Experimental	7	25.14
3043	Alltech Experimental	7	30.82

TABLE 10 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
3045	Alltech Experimental	7	19.77
3046	Alltech Experimental	7	20.18
3061	Alltech Experimental	7	19.14
3067	Alltech Experimental	7	20.05
1675	Control	11	10.82
1797	Control	11	6.55
1855	Control	11	24.14
1898	Control	11	18.09
1924	Control	11	21.91
1931	Control	11	23.91
1970	Control	11	18.32
1973	Control	11	19.86
1978	Control	11	26.23
3041	Control	11	28.95
3047	Control	11	20.09
3065	Control	11	20.14
1544	MTB-100 (2004)	11	24.14
1786	MTB-100 (2004)	11	11.91
1823	MTB-100 (2004)	11	5.73
1880	MTB-100 (2004)	11	16.86
1910	MTB-100 (2004)	11	10.73
1925	MTB-100 (2004)	11	19.45
1951	MTB-100 (2004)	11	26.64
1952	MTB-100 (2004)	11	22.68
1972	MTB-100 (2004)	11	19.77
1980	MTB-100 (2004)	11	16.82
1988	MTB-100 (2004)	11	16.27
3060	MTB-100 (2004)	11	21.50
1914	MTB-100 (2006)	11	20.09
1919	MTB-100 (2006)	11	13.55
1960	MTB-100 (2006)	11	19.91
1982	MTB-100 (2006)	11	23.14
1983	MTB-100 (2006)	11	25.73
2963	MTB-100 (2006)	11	24.05
3002	MTB-100 (2006)	11	11.82
3004	MTB-100 (2006)	11	18.32

TABLE 10 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
3031	MTB-100 (2006)	11	22.55
3033	MTB-100 (2006)	11	20.23
3068	MTB-100 (2006)	11	23.77
3069	MTB-100 (2006)	11	21.41
1881	Alltech Experimental	11	8.09
1890	Alltech Experimental	11	17.50
1904	Alltech Experimental	11	23.05
1961	Alltech Experimental	11	15.05
2992	Alltech Experimental	11	14.05
3022	Alltech Experimental	11	31.05
3026	Alltech Experimental	11	25.09
3043	Alltech Experimental	11	26.36
3045	Alltech Experimental	11	21.36
3046	Alltech Experimental	11	19.23
3061	Alltech Experimental	11	16.68
3067	Alltech Experimental	11	16.18
1675	Control	12	16.32
1797	Control	12	6.95
1855	Control	12	20.91
1898	Control	12	17.91
1924	Control	12	23.32
1931	Control	12	26.77
1970	Control	12	13.09
1973	Control	12	19.50
1978	Control	12	26.45
3041	Control	12	26.55
3047	Control	12	21.55
3065	Control	12	21.18
1544	MTB-100 (2004)	12	25.27
1786	MTB-100 (2004)	12	11.64
1823	MTB-100 (2004)	12	5.45
1880	MTB-100 (2004)	12	18.41
1910	MTB-100 (2004)	12	13.64
1925	MTB-100 (2004)	12	19.50
1951	MTB-100 (2004)	12	25.91
1952	MTB-100 (2004)	12	22.45

TABLE 10 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1972	MTB-100 (2004)	12	22.18
1980	MTB-100 (2004)	12	15.18
1988	MTB-100 (2004)	12	18.41
3060	MTB-100 (2004)	12	24.86
1914	MTB-100 (2006)	12	18.77
1919	MTB-100 (2006)	12	15.05
1960	MTB-100 (2006)	12	22.64
1982	MTB-100 (2006)	12	25.36
1983	MTB-100 (2006)	12	26.32
2963	MTB-100 (2006)	12	21.73
3002	MTB-100 (2006)	12	13.91
3004	MTB-100 (2006)	12	19.27
3031	MTB-100 (2006)	12	23.64
3033	MTB-100 (2006)	12	23.77
3068	MTB-100 (2006)	12	23.36
3069	MTB-100 (2006)	12	21.91
1881	Alltech Experimental	12	11.50
1890	Alltech Experimental	12	16.50
1904	Alltech Experimental	12	25.73
1961	Alltech Experimental	12	17.18
2992	Alltech Experimental	12	17.50
3022	Alltech Experimental	12	32.41
3026	Alltech Experimental	12	25.36
3043	Alltech Experimental	12	27.50
3045	Alltech Experimental	12	22.05
3046	Alltech Experimental	12	21.05
3061	Alltech Experimental	12	19.23
3067	Alltech Experimental	12	19.68
1675	Control	13	12.18
1797	Control	13	7.18
1855	Control	13	22.82
1898	Control	13	17.36
1924	Control	13	22.50
1931	Control	13	25.09
1970	Control	13	18.59
1973	Control	13	19.05

TABLE 10 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1978	Control	13	27.82
3041	Control	13	28.50
3047	Control	13	23.09
3065	Control	13	21.09
1544	MTB-100 (2004)	13	25.23
1786	MTB-100 (2004)	13	13.09
1823	MTB-100 (2004)	13	6.45
1880	MTB-100 (2004)	13	17.41
1910	MTB-100 (2004)	13	13.23
1925	MTB-100 (2004)	13	17.32
1951	MTB-100 (2004)	13	33.82
1952	MTB-100 (2004)	13	28.05
1972	MTB-100 (2004)	13	21.77
1980	MTB-100 (2004)	13	16.14
1988	MTB-100 (2004)	13	19.23
3060	MTB-100 (2004)	13	22.91
1914	MTB-100 (2006)	13	20.86
1919	MTB-100 (2006)	13	13.77
1960	MTB-100 (2006)	13	20.64
1982	MTB-100 (2006)	13	27.64
1983	MTB-100 (2006)	13	23.55
2963	MTB-100 (2006)	13	23.23
3002	MTB-100 (2006)	13	12.50
3004	MTB-100 (2006)	13	19.18
3031	MTB-100 (2006)	13	21.09
3033	MTB-100 (2006)	13	21.91
3068	MTB-100 (2006)	13	24.41
3069	MTB-100 (2006)	13	23.00
1881	Alltech Experimental	13	11.91
1890	Alltech Experimental	13	19.18
1904	Alltech Experimental	13	27.68
1961	Alltech Experimental	13	16.45
2992	Alltech Experimental	13	16.45
3022	Alltech Experimental	13	34.59
3026	Alltech Experimental	13	25.23
3043	Alltech Experimental	13	31.14

TABLE 10 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
3045	Alltech Experimental	13	21.27
3046	Alltech Experimental	13	20.14
3061	Alltech Experimental	13	19.86
3067	Alltech Experimental	13	19.27

TABLE 11. Experiment 3, milk yield (kg) from cows consuming approximately 80 to 100 ppb aflatoxin-contaminated diets without a sequestering agent (days 4 to 8) and with the inclusion of a sequestering agent (days 11 to 15), except for control that received no sequestering agent.

Cow No.	Treatment	Day	Milk yield (kg)
1833	Control	4	18.9
1904	Control	4	23.2
1983	Control	4	24.5
3041	Control	4	12.6
1747	MTB-100 (2006)	4	20.8
1762	MTB-100 (2006)	4	21.5
1828	MTB-100 (2006)	4	14.1
1891	MTB-100 (2006)	4	15.8
3043	MTB-100 (2006)	4	14.1
1753	AB-20A	4	24.0
1791	AB-20A	4	19.5
1803	AB-20A	4	14.4
1982	AB-20A	4	19.7
3032	AB-20A	4	23.4
1833	Control	5	19.1
1904	Control	5	24.3
1983	Control	5	27.1
3041	Control	5	18.4
1747	MTB-100 (2006)	5	15.1
1762	MTB-100 (2006)	5	23.5
1828	MTB-100 (2006)	5	18.4

TABLE 11 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1891	MTB-100 (2006)	5	18.3
3043	MTB-100 (2006)	5	16.1
1753	AB-20A	5	22.3
1791	AB-20A	5	19.8
1803	AB-20A	5	19.5
1982	AB-20A	5	21.1
3032	AB-20A	5	27.0
1833	Control	6	22.0
1904	Control	6	27.0
1983	Control	6	25.4
3041	Control	6	11.0
1747	MTB-100 (2006)	6	17.6
1762	MTB-100 (2006)	6	28.8
1828	MTB-100 (2006)	6	17.3
1891	MTB-100 (2006)	6	16.1
3043	MTB-100 (2006)	6	14.0
1753	AB-20A	6	23.3
1791	AB-20A	6	21.1
1803	AB-20A	6	17.1
1982	AB-20A	6	21.6
3032	AB-20A	6	25.6
1833	Control	7	19.9
1904	Control	7	23.0
1983	Control	7	22.5
3041	Control	7	.
1747	MTB-100 (2006)	7	18.4
1762	MTB-100 (2006)	7	24.0
1828	MTB-100 (2006)	7	14.0
1891	MTB-100 (2006)	7	18.9
3043	MTB-100 (2006)	7	13.4
1753	AB-20A	7	22.5
1791	AB-20A	7	16.5
1803	AB-20A	7	17.1
1982	AB-20A	7	21.0
3032	AB-20A	7	24.2
1833	Control	8	17.3

TABLE 11 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1904	Control	8	22.8
1983	Control	8	22.4
3041	Control	8	10.3
1747	MTB-100 (2006)	8	21.4
1762	MTB-100 (2006)	8	24.6
1828	MTB-100 (2006)	8	15.7
1891	MTB-100 (2006)	8	18.0
3043	MTB-100 (2006)	8	13.9
1753	AB-20A	8	23.4
1791	AB-20A	8	18.4
1803	AB-20A	8	17.0
1982	AB-20A	8	22.3
3032	AB-20A	8	22.5
1833	Control	11	19.2
1904	Control	11	22.0
1983	Control	11	24.6
3041	Control	11	9.0
1747	MTB-100 (2006)	11	18.9
1762	MTB-100 (2006)	11	29.6
1828	MTB-100 (2006)	11	14.6
1891	MTB-100 (2006)	11	19.8
3043	MTB-100 (2006)	11	13.1
1753	AB-20A	11	24.5
1791	AB-20A	11	17.3
1803	AB-20A	11	15.1
1982	AB-20A	11	36.8
3032	AB-20A	11	26.0
1833	Control	12	.
1904	Control	12	.
1983	Control	12	.
3041	Control	12	.
1747	MTB-100 (2006)	12	.
1762	MTB-100 (2006)	12	.
1828	MTB-100 (2006)	12	.
1891	MTB-100 (2006)	12	.
3043	MTB-100 (2006)	12	.

TABLE 11 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
1753	AB-20A	12	.
1791	AB-20A	12	.
1803	AB-20A	12	.
1982	AB-20A	12	.
3032	AB-20A	12	.
1833	Control	13	.
1904	Control	13	.
1983	Control	13	.
3041	Control	13	.
1747	MTB-100 (2006)	13	.
1762	MTB-100 (2006)	13	.
1828	MTB-100 (2006)	13	.
1891	MTB-100 (2006)	13	.
3043	MTB-100 (2006)	13	.
1753	AB-20A	13	.
1791	AB-20A	13	.
1803	AB-20A	13	.
1982	AB-20A	13	.
3032	AB-20A	13	.
1833	Control	14	19.4
1904	Control	14	23.5
1983	Control	14	25.1
3041	Control	14	8.68
1747	MTB-100 (2006)	14	18.3
1762	MTB-100 (2006)	14	27.1
1828	MTB-100 (2006)	14	14.8
1891	MTB-100 (2006)	14	21.0
3043	MTB-100 (2006)	14	11.3
1753	AB-20A	14	21.9
1791	AB-20A	14	16.2
1803	AB-20A	14	15.6
1982	AB-20A	14	21.7
3032	AB-20A	14	25.7
1833	Control	15	18.7
1904	Control	15	23.1
1983	Control	15	25.0

TABLE 11 (continued).

Cow No.	Treatment	Day in Trial	Milk Yield (kg)
3041	Control	15	7.00
1747	MTB-100 (2006)	15	22.1
1762	MTB-100 (2006)	15	28.5
1828	MTB-100 (2006)	15	15.7
1891	MTB-100 (2006)	15	16.7
3043	MTB-100 (2006)	15	11.0
1753	AB-20A	15	22.1
1791	AB-20A	15	18.0
1803	AB-20A	15	17.3
1982	AB-20A	15	22.3
3032	AB-20A	15	26.9

TABLE 12. Experiment 1, dry matter intake (kg) from cows consuming approximately 80 to 100 ppb aflatoxin-contaminated diets without a sequestering agent (days 4 to 8) and with the inclusion of a sequestering agent (days 11 to 15), except for control that received no sequestering agent.

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1675	Control	5	27.6
1797	Control	5	22.3
1855	Control	5	27.6
1898	Control	5	28.5
1924	Control	5	16.4
1931	Control	5	24.0
1970	Control	5	7.61
1973	Control	5	19.7
1978	Control	5	22.3.
3041	Control	5	27.6
3047	Control	5	23.4
3065	Control	5	20.9

TABLE 12 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1745	Lallemand	5	24.2
1763	Lallemand	5	24.2
1865	Lallemand	5	25.9
1905	Lallemand	5	25.1
1934	Lallemand	5	19.2
1936	Lallemand	5	17.8
1957	Lallemand	5	24.0
1963	Lallemand	5	21.7
1966	Lallemand	5	23.7
3023	Lallemand	5	22.6
3035	Lallemand	5	23.1
3063	Lallemand	5	22.6
1675	Control	6	27.0
1797	Control	6	24.3
1855	Control	6	29.5
1898	Control	6	33.3
1924	Control	6	13.6
1931	Control	6	26.4
1970	Control	6	12.8
1973	Control	6	19.0
1978	Control	6	16.6
3041	Control	6	28.3
3047	Control	6	23.7
3065	Control	6	23.4
1745	Lallemand	6	21.2
1763	Lallemand	6	26.4
1865	Lallemand	6	24.0
1905	Lallemand	6	25.3
1934	Lallemand	6	21.2
1936	Lallemand	6	22.0
1957	Lallemand	6	25.6
1963	Lallemand	6	23.4
1966	Lallemand	6	23.1
3023	Lallemand	6	28.6
3035	Lallemand	6	24.0
3063	Lallemand	6	23.1

TABLE 12 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1675	Control	7	28.4
1797	Control	7	24.5
1855	Control	7	26.6
1898	Control	7	33.2
1924	Control	7	22.4
1931	Control	7	25.0
1970	Control	7	18.1
1973	Control	7	18.4
1978	Control	7	23.7
3041	Control	7	28.7
3047	Control	7	23.9
3065	Control	7	21.8
1745	Lallemand	7	21.3
1763	Lallemand	7	22.6
1865	Lallemand	7	25.3
1905	Lallemand	7	26.3
1934	Lallemand	7	18.7
1936	Lallemand	7	23.4
1957	Lallemand	7	25.0
1963	Lallemand	7	24.7
1966	Lallemand	7	26.3
3023	Lallemand	7	27.9
3035	Lallemand	7	23.7
3063	Lallemand	7	18.9
1675	Control	11	25.9
1797	Control	11	25.0
1855	Control	11	29.1
1898	Control	11	32.4
1924	Control	11	25.9
1931	Control	11	26.4
1970	Control	11	18.5
1973	Control	11	21.5
1978	Control	11	23.1
3041	Control	11	27.8
3047	Control	11	24.5
3065	Control	11	21.0

TABLE 12 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1745	Lallemand	11	25.9
1763	Lallemand	11	24.8
1865	Lallemand	11	29.1
1905	Lallemand	11	25.6
1934	Lallemand	11	20.4
1936	Lallemand	11	22.0
1957	Lallemand	11	26.7
1963	Lallemand	11	24.8
1966	Lallemand	11	28.3
3023	Lallemand	11	15.2
3035	Lallemand	11	23.7
3063	Lallemand	11	24.0
1675	Control	12	28.1
1797	Control	12	27.6
1855	Control	12	28.4
1898	Control	12	28.6
1924	Control	12	26.2
1931	Control	12	28.4
1970	Control	12	17.4
1973	Control	12	22.2
1978	Control	12	25.9
3041	Control	12	29.5
3047	Control	12	24.8
3065	Control	12	22.8
1745	Lallemand	12	26.1
1763	Lallemand	12	28.9
1865	Lallemand	12	26.3
1905	Lallemand	12	27.3
1934	Lallemand	12	21.9
1936	Lallemand	12	24.6
1957	Lallemand	12	26.4
1963	Lallemand	12	25.8
1966	Lallemand	12	27.4
3023	Lallemand	12	28.1
3035	Lallemand	12	24.7
3063	Lallemand	12	22.3

TABLE 12 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1675	Control	13	28.6
1797	Control	13	21.0
1855	Control	13	24.5
1898	Control	13	34.3
1924	Control	13	21.5
1931	Control	13	27.8
1970	Control	13	13.9
1973	Control	13	20.1
1978	Control	13	23.1
3041	Control	13	22.3
3047	Control	13	22.9
3065	Control	13	22.6
1745	Lallemand	13	25.7
1763	Lallemand	13	22.7
1865	Lallemand	13	25.5
1905	Lallemand	13	26.0
1934	Lallemand	13	17.1
1936	Lallemand	13	21.6
1957	Lallemand	13	27.7
1963	Lallemand	13	23.0
1966	Lallemand	13	24.1
3023	Lallemand	13	29.9
3035	Lallemand	13	23.8
3063	Lallemand	13	17.1

TABLE 13. Experiment 2, dry matter intake (kg) from cows consuming approximately 80 to 100 ppb aflatoxin-contaminated diets without a sequestering agent (days 4 to 8) and with the inclusion of a sequestering agent (days 11 to 15), except for control that received no sequestering agent.

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1675	Control	5	27.6
1797	Control	5	22.2
1855	Control	5	27.6
1898	Control	5	28.4
1924	Control	5	16.3
1931	Control	5	23.9
1970	Control	5	7.6
1973	Control	5	19.7
1978	Control	5	22.2
3041	Control	5	27.6
3047	Control	5	23.3
3065	Control	5	20.8
1544	MTB-100 (2004)	5	28.7
1786	MTB-100 (2004)	5	18.3
1823	MTB-100 (2004)	5	21.7
1880	MTB-100 (2004)	5	19.1
1910	MTB-100 (2004)	5	20.0
1925	MTB-100 (2004)	5	22.2
1951	MTB-100 (2004)	5	25.0
1952	MTB-100 (2004)	5	26.2
1972	MTB-100 (2004)	5	24.8
1980	MTB-100 (2004)	5	20.5
1988	MTB-100 (2004)	5	15.5
3060	MTB-100 (2004)	5	16.0
1914	MTB-100 (2006)	5	22.5
1919	MTB-100 (2006)	5	24.8
1960	MTB-100 (2006)	5	21.1
1982	MTB-100 (2006)	5	26.7
1983	MTB-100 (2006)	5	22.2
2963	MTB-100 (2006)	5	25.3
3002	MTB-100 (2006)	5	21.1
3004	MTB-100 (2006)	5	11.5

TABLE 13 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
3031	MTB-100 (2006)	5	23.9
3033	MTB-100 (2006)	5	27.3
3068	MTB-100 (2006)	5	18.6
3069	MTB-100 (2006)	5	21.4
1881	Alltech Experimental	5	22.2
1890	Alltech Experimental	5	27.9
1904	Alltech Experimental	5	22.5
1961	Alltech Experimental	5	21.9
2992	Alltech Experimental	5	21.7
3022	Alltech Experimental	5	28.4
3026	Alltech Experimental	5	27.3
3043	Alltech Experimental	5	25.9
3045	Alltech Experimental	5	22.5
3046	Alltech Experimental	5	16.9
3061	Alltech Experimental	5	23.1
3067	Alltech Experimental	5	22.8
1675	Control	6	27.0
1797	Control	6	24.2
1855	Control	6	29.4
1898	Control	6	33.2
1924	Control	6	13.6
1931	Control	6	26.4
1970	Control	6	12.8
1973	Control	6	19.0
1978	Control	6	16.6
3041	Control	6	28.3
3047	Control	6	23.7
3065	Control	6	23.4
1544	MTB-100 (2004)	6	27.5
1786	MTB-100 (2004)	6	19.9
1823	MTB-100 (2004)	6	24.0
1880	MTB-100 (2004)	6	16.9
1910	MTB-100 (2004)	6	23.7
1925	MTB-100 (2004)	6	27.8
1951	MTB-100 (2004)	6	24.5
1952	MTB-100 (2004)	6	24.8

TABLE 13 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1972	MTB-100 (2004)	6	26.1
1980	MTB-100 (2004)	6	22.3
1988	MTB-100 (2004)	6	20.4
3060	MTB-100 (2004)	6	21.8
1914	MTB-100 (2006)	6	23.7
1919	MTB-100 (2006)	6	26.4
1960	MTB-100 (2006)	6	23.4
1982	MTB-100 (2006)	6	28.3
1983	MTB-100 (2006)	6	25.3
2963	MTB-100 (2006)	6	33.0
3002	MTB-100 (2006)	6	28.6
3004	MTB-100 (2006)	6	24.0
3031	MTB-100 (2006)	6	30.0
3033	MTB-100 (2006)	6	29.73
3068	MTB-100 (2006)	6	22.6
3069	MTB-100 (2006)	6	24.0
1881	Alltech Experimental	6	24.0
1890	Alltech Experimental	6	33.8
1904	Alltech Experimental	6	24.2
1961	Alltech Experimental	6	23.1
2992	Alltech Experimental	6	25.6
3022	Alltech Experimental	6	15.0
3026	Alltech Experimental	6	27.5
3043	Alltech Experimental	6	29.7
3045	Alltech Experimental	6	23.7
3046	Alltech Experimental	6	19.9
3061	Alltech Experimental	6	25.6
3067	Alltech Experimental	6	25.6
1675	Control	7	28.4
1797	Control	7	24.5
1855	Control	7	26.6
1898	Control	7	33.2
1924	Control	7	22.4
1931	Control	7	25.0
1970	Control	7	18.1
1973	Control	7	18.4

TABLE 13 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1978	Control	7	23.7
3041	Control	7	28.7
3047	Control	7	23.9
3065	Control	7	21.8
1544	MTB-100 (2004)	7	27.4
1786	MTB-100 (2004)	7	21.6
1823	MTB-100 (2004)	7	21.3
1880	MTB-100 (2004)	7	18.7
1910	MTB-100 (2004)	7	21.3
1925	MTB-100 (2004)	7	29.2
1951	MTB-100 (2004)	7	25.5
1952	MTB-100 (2004)	7	27.4
1972	MTB-100 (2004)	7	25.3
1980	MTB-100 (2004)	7	19.7
1988	MTB-100 (2004)	7	21.0
3060	MTB-100 (2004)	7	25.0
1914	MTB-100 (2006)	7	24.5
1919	MTB-100 (2006)	7	24.5
1960	MTB-100 (2006)	7	21.3
1982	MTB-100 (2006)	7	27.9
1983	MTB-100 (2006)	7	23.4
2963	MTB-100 (2006)	7	29.2
3002	MTB-100 (2006)	7	30.3
3004	MTB-100 (2006)	7	23.9
3031	MTB-100 (2006)	7	30.8
3033	MTB-100 (2006)	7	27.4
3068	MTB-100 (2006)	7	22.1
3069	MTB-100 (2006)	7	26.8
1881	Alltech Experimental	7	16.6
1890	Alltech Experimental	7	30.5
1904	Alltech Experimental	7	24.2
1961	Alltech Experimental	7	21.6
2992	Alltech Experimental	7	21.6
3022	Alltech Experimental	7	27.6
3026	Alltech Experimental	7	28.4
3043	Alltech Experimental	7	26.8

TABLE 13 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
3045	Alltech Experimental	7	24.5
3046	Alltech Experimental	7	17.1
3061	Alltech Experimental	7	19.7
3067	Alltech Experimental	7	23.9
1675	Control	11	25.9
1797	Control	11	25.0
1855	Control	11	29.1
1898	Control	11	32.4
1924	Control	11	25.9
1931	Control	11	26.4
1970	Control	11	18.5
1973	Control	11	21.5
1978	Control	11	23.1
3041	Control	11	27.8
3047	Control	11	24.5
3065	Control	11	21.0
1544	MTB-100 (2004)	11	29.6
1786	MTB-100 (2004)	11	18.8
1823	MTB-100 (2004)	11	24.1
1880	MTB-100 (2004)	11	19.9
1910	MTB-100 (2004)	11	26.3
1925	MTB-100 (2004)	11	29.1
1951	MTB-100 (2004)	11	26.6
1952	MTB-100 (2004)	11	25.5
1972	MTB-100 (2004)	11	30.5
1980	MTB-100 (2004)	11	17.1
1988	MTB-100 (2004)	11	21.0
3060	MTB-100 (2004)	11	18.5
1914	MTB-100 (2006)	11	24.5
1919	MTB-100 (2006)	11	25.9
1960	MTB-100 (2006)	11	23.1
1982	MTB-100 (2006)	11	25.6
1983	MTB-100 (2006)	11	24.8
2963	MTB-100 (2006)	11	29.4
3002	MTB-100 (2006)	11	30.8
3004	MTB-100 (2006)	11	25.6

TABLE 13 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
3031	MTB-100 (2006)	11	26.4
3033	MTB-100 (2006)	11	29.1
3068	MTB-100 (2006)	11	21.2
3069	MTB-100 (2006)	11	23.4
1881	Alltech Experimental	11	24.4
1890	Alltech Experimental	11	29.9
1904	Alltech Experimental	11	26.3
1961	Alltech Experimental	11	21.9
2992	Alltech Experimental	11	22.1
3022	Alltech Experimental	11	33.0
3026	Alltech Experimental	11	28.2
3043	Alltech Experimental	11	29.1
3045	Alltech Experimental	11	26.0
3046	Alltech Experimental	11	19.4
3061	Alltech Experimental	11	25.2
3067	Alltech Experimental	11	24.6
1675	Control	12	28.1
1797	Control	12	27.6
1855	Control	12	28.4
1898	Control	12	36.6
1924	Control	12	26.2
1931	Control	12	28.4
1970	Control	12	17.4
1973	Control	12	22.2
1978	Control	12	25.9
3041	Control	12	29.5
3047	Control	12	24.8
3065	Control	12	22.8
1544	MTB-100 (2004)	12	29.0
1786	MTB-100 (2004)	12	18.0
1823	MTB-100 (2004)	12	25.6
1880	MTB-100 (2004)	12	16.0
1910	MTB-100 (2004)	12	25.9
1925	MTB-100 (2004)	12	29.0
1951	MTB-100 (2004)	12	28.7
1952	MTB-100 (2004)	12	26.4

TABLE 13 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1972	MTB-100 (2004)	12	32.4
1980	MTB-100 (2004)	12	18.3
1988	MTB-100 (2004)	12	22.2
3060	MTB-100 (2004)	12	23.1
1914	MTB-100 (2006)	12	23.0
1919	MTB-100 (2006)	12	23.1
1960	MTB-100 (2006)	12	19.6
1982	MTB-100 (2006)	12	20.2
1983	MTB-100 (2006)	12	20.4
2963	MTB-100 (2006)	12	27.6
3002	MTB-100 (2006)	12	21.6
3004	MTB-100 (2006)	12	20.2
3031	MTB-100 (2006)	12	25.4
3033	MTB-100 (2006)	12	24.7
3068	MTB-100 (2006)	12	18.9
3069	MTB-100 (2006)	12	21.5
1881	Alltech Experimental	12	22.0
1890	Alltech Experimental	12	27.8
1904	Alltech Experimental	12	26.7
1961	Alltech Experimental	12	21.2
2992	Alltech Experimental	12	20.1
3022	Alltech Experimental	12	32.1
3026	Alltech Experimental	12	30.0
3043	Alltech Experimental	12	26.7
3045	Alltech Experimental	12	26.7
3046	Alltech Experimental	12	18.8
3061	Alltech Experimental	12	10.9
3067	Alltech Experimental	12	24.2
1675	Control	13	28.6
1797	Control	13	21.0
1855	Control	13	24.5
1898	Control	13	34.3
1924	Control	13	21.5
1931	Control	13	27.8
1970	Control	13	13.9
1973	Control	13	20.1

TABLE 13 (continued).

Cow No.	Treatment	Day inTrial	Dry Matter Intake (kg)
1978	Control	13	23.1
3041	Control	13	22.3
3047	Control	13	22.9
3065	Control	13	22.6
1544	MTB-100 (2004)	13	26.9
1786	MTB-100 (2004)	13	20.8
1823	MTB-100 (2004)	13	23.0
1880	MTB-100 (2004)	13	13.0
1910	MTB-100 (2004)	13	24.4
1925	MTB-100 (2004)	13	27.1
1951	MTB-100 (2004)	13	23.2
1952	MTB-100 (2004)	13	26.3
1972	MTB-100 (2004)	13	30.2
1980	MTB-100 (2004)	13	16.0
1988	MTB-100 (2004)	13	22.1
3060	MTB-100 (2004)	13	15.5
1914	MTB-100 (2006)	13	21.3
1919	MTB-100 (2006)	13	18.0
1960	MTB-100 (2006)	13	19.9
1982	MTB-100 (2006)	13	23.8
1983	MTB-100 (2006)	13	26.0
2963	MTB-100 (2006)	13	26.3
3002	MTB-100 (2006)	13	26.6
3004	MTB-100 (2006)	13	26.0
3031	MTB-100 (2006)	13	28.0
3033	MTB-100 (2006)	13	26.9
3068	MTB-100 (2006)	13	19.6
3069	MTB-100 (2006)	13	20.2
1881	Alltech Experimental	13	20.8
1890	Alltech Experimental	13	24.9
1904	Alltech Experimental	13	26.6
1961	Alltech Experimental	13	19.4
2992	Alltech Experimental	13	22.1
3022	Alltech Experimental	13	23.5
3026	Alltech Experimental	13	28.5
3043	Alltech Experimental	13	6.9

TABLE 13 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
3045	Alltech Experimental	13	15.8
3046	Alltech Experimental	13	16.9
3061	Alltech Experimental	13	24.9
3067	Alltech Experimental	13	24.9

TABLE 14. Experiment 3, dry matter intake (kg) from cows consuming approximately 80 to 100 ppb aflatoxin-contaminated diets without a sequestering agent (days 4 to 8) and with the inclusion of a sequestering agent (days 11 to 15), except for control that received no sequestering agent.

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1833	Control	4	26.8
1904	Control	4	25.2
1983	Control	4	28.1
3041	Control	4	23.1
1747	MTB-100 (2006)	4	15.1
1762	MTB-100 (2006)	4	20.8
1828	MTB-100 (2006)	4	26.0
1891	MTB-100 (2006)	4	24.4
3043	MTB-100 (2006)	4	24.1
1753	AB-20A	4	28.9
1791	AB-20A	4	24.1
1803	AB-20A	4	25.4
1982	AB-20A	4	20.8
3032	AB-20A	4	27.5
1833	Control	5	24.3
1904	Control	5	23.4
1983	Control	5	21.1
3041	Control	5	9.85
1747	MTB-100 (2006)	5	19.8
1762	MTB-100 (2006)	5	22.3

TABLE 14 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1828	MTB-100 (2006)	5	23.4
1891	MTB-100 (2006)	5	24.6
3043	MTB-100 (2006)	5	22.3
1753	AB-20A	5	27.2
1791	AB-20A	5	22.3
1803	AB-20A	5	22.9
1982	AB-20A	5	22.2
3032	AB-20A	5	25.0
1833	Control	6	23.4
1904	Control	6	23.7
1983	Control	6	24.0
3041	Control	6	8.79
1747	MTB-100 (2006)	6	17.0
1762	MTB-100 (2006)	6	23.1
1828	MTB-100 (2006)	6	23.4
1891	MTB-100 (2006)	6	24.3
3043	MTB-100 (2006)	6	21.4
1753	AB-20A	6	25.8
1791	AB-20A	6	25.5
1803	AB-20A	6	19.6
1982	AB-20A	6	24.0
3032	AB-20A	6	27.5
1833	Control	7	21.3
1904	Control	7	22.7
1983	Control	7	21.0
3041	Control	7	9.65
1747	MTB-100 (2006)	7	15.9
1762	MTB-100 (2006)	7	23.0
1828	MTB-100 (2006)	7	23.2
1891	MTB-100 (2006)	7	22.1
3043	MTB-100 (2006)	7	21.3
1753	AB-20A	7	19.8
1791	AB-20A	7	25.8
1803	AB-20A	7	18.4
1982	AB-20A	7	23.8
3032	AB-20A	7	26.4

TABLE 14 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1833	Control	8	23.7
1904	Control	8	25.2
1983	Control	8	25.2
3041	Control	8	8.22
1747	MTB-100 (2006)	8	20.1
1762	MTB-100 (2006)	8	25.5
1828	MTB-100 (2006)	8	26.4
1891	MTB-100 (2006)	8	26.8
3043	MTB-100 (2006)	8	24.3
1753	AB-20A	8	26.8
1791	AB-20A	8	29.2
1803	AB-20A	8	22.8
1982	AB-20A	8	25.5
3032	AB-20A	8	29.8
1833	Control	9	20.8
1904	Control	9	23.8
1983	Control	9	26.0
3041	Control	9	12.7
1747	MTB-100 (2006)	9	19.8
1762	MTB-100 (2006)	9	23.4
1828	MTB-100 (2006)	9	24.6
1891	MTB-100 (2006)	9	25.3
3043	MTB-100 (2006)	9	25.7
1753	AB-20A	9	24.1
1791	AB-20A	9	23.1
1803	AB-20A	9	23.3
1982	AB-20A	9	23.9
3032	AB-20A	9	29.3
1833	Control	10	23.0
1904	Control	10	22.9
1983	Control	10	26.0
3041	Control	10	12.9
1747	MTB-100 (2006)	10	16.3
1762	MTB-100 (2006)	10	25.5
1828	MTB-100 (2006)	10	25.1
1891	MTB-100 (2006)	10	24.7

TABLE 14 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
3043	MTB-100 (2006)	10	21.7
1753	AB-20A	10	26.1
1791	AB-20A	10	25.0
1803	AB-20A	10	23.0
1982	AB-20A	10	20.5
3032	AB-20A	10	20.0
1833	Control	11	21.2
1904	Control	11	22.9
1983	Control	11	23.1
3041	Control	11	13.3
1747	MTB-100 (2006)	11	19.7
1762	MTB-100 (2006)	11	23.3
1828	MTB-100 (2006)	11	23.7
1891	MTB-100 (2006)	11	23.7
3043	MTB-100 (2006)	11	24.2
1753	AB-20A	11	24.9
1791	AB-20A	11	24.6
1803	AB-20A	11	21.3
1982	AB-20A	11	24.2
3032	AB-20A	11	26.8
1833	Control	12	22.9
1904	Control	12	24.9
1983	Control	12	25.9
3041	Control	12	16.6
1747	MTB-100 (2006)	12	18.9
1762	MTB-100 (2006)	12	25.0
1828	MTB-100 (2006)	12	25.1
1891	MTB-100 (2006)	12	23.6
3043	MTB-100 (2006)	12	25.0
1753	AB-20A	12	24.4
1791	AB-20A	12	25.7
1803	AB-20A	12	22.7
1982	AB-20A	12	24.9
3032	AB-20A	12	27.0
1833	Control	13	25.9
1904	Control	13	25.9

TABLE 14 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1983	Control	13	28.3
3041	Control	13	18.2
1747	MTB-100 (2006)	13	20.9
1762	MTB-100 (2006)	13	25.0
1828	MTB-100 (2006)	13	27.4
1891	MTB-100 (2006)	13	24.5
3043	MTB-100 (2006)	13	23.2
1753	AB-20A	13	26.0
1791	AB-20A	13	27.6
1803	AB-20A	13	22.8
1982	AB-20A	13	22.9
3032	AB-20A	13	28.3
1833	Control	14	23.4
1904	Control	14	23.6
1983	Control	14	25.4
3041	Control	14	22.0
1747	MTB-100 (2006)	14	18.9
1762	MTB-100 (2006)	14	24.4
1828	MTB-100 (2006)	14	24.7
1891	MTB-100 (2006)	14	24.0
3043	MTB-100 (2006)	14	25.4
1753	AB-20A	14	26.3
1791	AB-20A	14	23.8
1803	AB-20A	14	21.0
1982	AB-20A	14	25.3
3032	AB-20A	14	30.0
1833	Control	15	21.4
1904	Control	15	23.6
1983	Control	15	21.2
3041	Control	15	22.1
1747	MTB-100 (2006)	15	18.6
1762	MTB-100 (2006)	15	21.0
1828	MTB-100 (2006)	15	23.0
1891	MTB-100 (2006)	15	20.0
3043	MTB-100 (2006)	15	23.4
1753	AB-20A	15	26.4

TABLE 14 (continued).

Cow No.	Treatment	Day in Trial	Dry Matter Intake (kg)
1791	AB-20A	15	28.5
1803	AB-20A	15	21.4
1982	AB-20A	15	22.6
3032	AB-20A	15	24.9