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Reduction of Toxic Effects of Aflatoxin B_1 by Using Baker Yeast (*Saccharomyces cerevisiae*) in Growing Broiler Chicks Diets

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ABSTRACT - This study was carried out to investigate the effects of adding baker yeast (BY), chlortetracycline (CTC) and both BY + CTC to a control diet containing 200 ng/g of aflatoxin B_1 ($C + AFB_1$) on performance, serum parameters and pathologyc alterations of broilers. A total 100 chicks (Ross PM3) were divided into five groups in individual cages and each containing 20 animals. BY, a rich source of protein and vitamin B complex, was mixed into the diets at 2.0%, CTC was mixed into the diet at 2.5 ng/g. Feed consumption, body weight and feed efficiency were recorded weekly. Serum parameters and pathologyc alterations were determined at the end of the study. Dead animals were recorded daily. Liver changes were clearly apparent in the $C+AFB_1$ and $C+AFB_1$+CTC most of the livers were enlarged, yellow and had pethecial hemorrhages. Canalicula cholestosis was absent in group $C+AFB_1$ and $C+AFB_1$+CTC, but not others. When compared to the control (C) group, alkaline phosphatase (ALP), appear to be significantly increased in the $C+AFB_1$ and $C+CTC+AFB_1$ groups. Serum glutamic oxalacetic transaminase (GOT) was increased in $C+AFB_1$ birds. Serum alphaphetoprotein was not affected by the treatments. Feed consumption and body weight were significantly reduced in group AFB_1. Birds receiving $BY + AFB_1$, CTC + AFB_1 and $BY + CTC + AFB_1$ had a significantly higher body weight than group $C+AFB_1$. Feed efficiency was better in group $CTC + AFB_1$ than the others. The findings of this research suggest tha BY (2%) can partly counteract some of the toxic effects of AFB_1.

Key Words: feed, aflatoxin B_1, baker yeast, chlortetracycline, broiler, performance

Redução dos Efeitos Tóxicos da Aflatoxina B_1, Utilizando-se Levedura de Panificação (*Saccharomyces cerevisiae*), na Dieta de Pintos de Corte em Crescimento

RESUMO - Este estudo foi desenvolvido para avaliar os efeitos da adição de Levedura de panificação (BY) e cortetraciclina (CTC) e ambos BY+CTC a uma dieta controle © contendo 200 ng/g de aflatoxina B1 (C+AFB1) sobre desempenho, parâmetros séricos e alterações patológicas de frangos de corte. Um total de 100 pintinhos (Ross PM3) foi dividido em cinco grupos, em gaiolas individuais, contendo 20 animais para cada grupo. A levedura de panificação, uma fonte rica de proteína e vitamina do complexo B foi adicionada à dieta, à porcentagem de 2% e a CTC, a 2.5 ng/g. Consumo alimentar, peso corporal e eficiência alimentar foram registrados semanalmente. Parâmetros séricos e alterações patológicas foram determinados no final do estudo. Animais mortos foram registrados diariamente. Alterações ocorridas no fígado foram claramente aparentes nas dietas C+AFB1 e C+AFB1+CTC, sendo que a maioria dos fígados apresentou-se aumentado, amarelo e com hemorragia peticial. Canícula colestoses foi ausente nos grupos C+AFB1 e C+AFB1=CTC, mas não nos demais grupos. Quando comparado com o grupo controle©, alcalino fosfatase (ALP), parece que houve aumento significativo nos grupos C+AFB1 e C=CTC=AFB1. O xaloacético tansaminase glutâmico (GOT) sérico foi maior no grupo de aves C+AFB1. A lphafetoproteína sérica não foi afetada pelos tratamentos. Consumo alimentar e peso corporal foram significativamente reduzidos no grupo AFB1. Animais que receberam BY+AFB1, CTC+AFB1 e BY+CTC+AFB1 tiveram aumento significativo no peso corporal, comparado com o grupo C+AFB1. A eficiência alimentar foi melhor no grupo CTC+AFB1 em comparação aos demais. Os resultados desta pesquisa sugerem que BY (2%) pode parcialmente neutralizar alguns dos efeitos tóxicos do AFB1.

Palavras-chave: alimento, aflatoxina B_1, levedura de panificação, chlortetracycline, frango de corte, desempenho

Introduction

The occurrence of mycotoxins in foods and feeds is a problem of major concern in all over the world. Profitability of poultry production can be greatly affected due to the frequency of feed contamination and the detrimental effects of these toxins on the performance (Hamilton, 1984). Aflatoxins, a group of closely related and biologycally active mycotoxins, are produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus*. They commonly occur as natural contaminant of poultry feeds (Edds & Bortell, 1983). Domestic animal species such as chickens, ducks, cattle, swine and turkeys consuming sublethal doses of aflatoxins for several days developed a toxic syndrome in which liver damage was the most significant change. According to Who (1979), the biological effects of aflatoxins could be categorized into two groups, long term and short term effects. Long term effects included chronic toxicity, cancer,

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birth defects and genetic alterations (Hayes, 1978). Aflatoxins affected all poultry species, although they generally take relatively high levels to cause mortality, low levels can be detrimental if continually fed. Young poultry, especially ducks and turkeys, are very susceptible. As a general rule, growing poultry should not receive more than 20 µg of aflatoxin in the diet. However, feeding levels lower than 20 µg may still reduce their resistance to disease, decrease their ability to withstand stress and bruising, and generally make them unthrifty (Coelho, 1990). Numerous strategies for the detoxification-inactivation of mycotoxins contaminated feed have been proposed (e.g., physical separation, thermal inactivation, irridiation, microbial degradation and treatment with a variety of chemicals). Pratical methods to detoxify aflatoxins contaminated feed on a large scale and in a cost-effective manner are not current (Norag, 1995). A new approach to the detoxify of aflatoxin is the use of organic and inorganic adsorptive and high protein compounds in the diet of farm animals and one of these organic material is baker yeast (*Saccharomyces cerevisiae*). Yeasts have been fed to animals for more than a hundred years, and commercial yeast products specifically produced for animal feeding (Reed & Nagodawýthana, 1991). Few species of yeast are commercially used. *Saccharomyces cerevisiae,* also known as ''bakers yeast'', is one of the most widely commercialized species and one of the effective adsorbent, rich in protein (40-45%), whose biological value is high and is also rich in vitamin B complex. Several vitamins were first extracted and characterized from yeast, including biotin, niacin, pantothenic acid and thiamin (Reed & Nagodawýthhana, 1991). With the advent of using yeast cultures as growth promoters in poultry diets, several benefical effects have been recorded.Mannan oligosaccharide (MOS) is a product designed to influence microbial ecology. It is derived from yeast cell walls and it consists primarily on phosphorylated glucomannans. Two modes of action are now recognized: (1) It binds to lectins on the cell walls of certain undesirable bacteria. These bacterial lectins normally bind to the intestinal epithelial cells and aid the bacteria in colonization of the gut. However, if the lectins are bound to MOS, they can not bind to the epithelial cells and undesirable bacteria are eliminated from the gut lumen. (2) It enhances certain actions of the immune system. These modes of action enable MOS to help protecting animals from pathogens. On the other hand, chlorotetracycline is a therapeutic antibiotic in poultry, effective in controlling signs of synovitis (Olson, 1976), in reducing numbers of *Clostridium perfringens* isolated from the ceca of birds infected with *Eimeria tenella* (Arakawa, 1975) and in reducing shed of *Salmonella typhimurium* in turkey poults (Nývas, 1976). Due to AFB_1 does increase the susceptibility of chicks to infections, antibiotics would likely be given to birds experiencing an infection along with a mycotoxicosis. This study was conducted to determine the effect of dietary AFB_1 (200 ng/g) when given baker yeast and CTC in growing broiler chicks.

Material and Methods

One hundred Ross PM 3 (5 treatments of 20 animals each) day-old male broiler chicks were obtained from a commercial hatchery, individually weighed, wing-banded and housed in experiment room and continuous fluorescent lighting. The birds were randomly assigned to the following treatment groups. Control diet without additives (treatment 1), aflatoxin B_1 AFB_1 (treatment 2), baker yeast (BY 3,44 x 10^8 CFU/g for 37ºC of 3 days) +AFB_1 (treatment 3), chlortetracycline (CTC)+AFB_1 (treatment 4) and BY+CTC+AFB_1 (treatment 5). Feed and water were provided for *ad libitum* consumption. Chicks were reared in individual wire cages for 37 days and fed a typical broiler diet with 22.0% crude protein and 12.8 metabolizable energy (MJ/kg) for starter phase (1-28 days) and 18.1% crude protein and 13.4 MJ/kg for growing phase (28-37 days). Diets were designed to satisfy the recommendations of the NRC (1984). BY and CTC were calculated and 2 kg per t and 2.5 ng/g feed, respectively, were added. The toxin was measured by spectrophotometric methods and it was estinated to be 200 ng/g. Feeds were analyzed for aflatoxins by thin layer chromatography, according to Howel (1983). Feed consumption efficiency and body weight were weekly determined. Dead animals were daily recorded. All chicks were sacrificed at the end of 37 days and 3-4 mL blood sample was taken and stored frozen at (-20ºC) until asseyed for blood enzymes, such as Glutamic Pyruvic Transaminase (GPT), Glutamic-Oxalacetic Transaminase (GOT), Alkaline Phosphatase (ALP) and Alphapheto protein (AFP). These parameters were evaluated by a clinical laboratory by SNA-12 method (Anonymous, 1974). Liver portions were checked by routinly tissueanalyses method. Pure crystalline AFB_1 was obtained from Sigma-Makor Chemical Corp., Jeusalem-Israel. AFB_1 was weighed and dissolved in warmed chloroform

under a hood. Feed and water were fed *ad libitum* until the end of study. Statistical variables, except for mortality and feed efficiency, that were evaluated by SAS (1986). Broiler chicks were daily examined in the morning and at evening for any sign of toxicosis and mortality. Liver portions (10 g) were collected from all sacrificed broiler chicks and frozen for analysis of mycotoxicosis and pathological examinations.

Results and Discussion

Data presented in Table 1 showed the effect of AFB_1 baker yeast, chlortetracycline and the combination on feed intake, feed efficiency and body weight, serum GOT, GPT, ALP and AFP of broilers. Feed intake and body weights of broilers receiving AFB_1 for the entire period (5 weeks) were significantly decreased in treatment 2 and increased in treatment 3 ($P<.05$). The birds that received AFB_1 at 200 ng/g level in treatment 2 had a significantly lower average body weight than others ($P<.05$). The birds fed dietary toxins with BY had a significantly higher feed intake than others ($P<.05$). Devedowda et al. (1997) clearly indicated the benifical effects of viable yeast culture when supplemented to aflatoxin-contaminated diets in poultry. Besides the positive effects on body weight, feed effciency and mortality, the most significant contribution was on the ability to modify immune response, which was reflected in the improvement in size of the bursa of Fabricus and increased levels of serum protein and albumin, thereby enhancing the levels of circulating immunoglobulins. In this research AFB_1 resulted in a significant decrease both feed intake and body weight in treatment 2 ($P<.05$). Birds receiving AFB_1 200 ng/g dietary in treatments 3, 4 and 5 had a significantly higher body weight than in treatment 1 and 2 ($P<.05$). Feed efficiency was better in treatment 4 than others. There was no mortalities attributed to AFB_1 in the 37 days of experiment in any of the groups. Control chickens were free of gross lesions and histologycally sound. At the end of study post- mortem examinations were performed on a total of 100 birds 8 from each group were removed at 37 days of age. Changes were clearly apparent in the livers of treatments 2 and 4. Most of livers were yellow and had pethecial hemorrhages and had enlarged. Liver section from birds group receiving 200 ng/g AFB_1 treatments 2 and 1 chicks showed individual hepatocytes to be swollen and fatty degenerations. The livers were swollen and congested in treatments 2 and 4. In the AFB_1 group of broilers acute hepatitis was observed. This histologic pattern was characterized by hepatocyte injury and necrosis accompained to varying degrees by lobular and portal inflammation. The mildest alterations consisted on scattered acidophilic bodies and foci of hepatocyte necrosis with minor inflammatory infiltration, yielding a picture of nonspecific reactive hepatitis. More substantial involvement yielded a distinctive viral hepatitis like appearence, diffuse lobular disarray with liver cell damage in the form of ballooning and acidophilic generation, spotty hepatocyte necrosis and variable inflammatory infiltration, predominantly by mononuclear cells. Canalicula cholestosis was absent in treatments 2 and 4, but not others. ALP appears to be significantly increased in the treatments 2 and 4 ($P<.05$).

Table 1 - Composition of the experimental diets fed to broiler chickens

Ingredients (g/kg)	Starter	Growing
Maize	350	500
Sorghum	200	170
Soybean meal[1]	336	221
Wheat	34	50
Fish meal [2]	50	30
Dicalcium phosphate	16	19
Ground limestone	7	5.0
Sodium chloride	2.5	2.5
DL-methionine	2	-
Vitamin mix	2.5	1.5
Mineral mix	1.0	1.0
Calculated composition		
Metabolizable energy (MJ/kg)	12.8	13.4
Crude protein (g/kg)	220	181

[1]Containing 446 g/kg protein.
[2]Containing 650 g/kg protein.

Serum glutamic oxalacetic transaminase (GOT) had increased ($P<.05$) in treatments 2, 3 and 4. Serum glutamic pyruvic transaminase (GPT) significantly increased ($P<.05$) in treatments 2, 3 and 4. No statistical differences were found between groups for serum AFP levels in all groups ($P<.05$). Considerably research had demonstrated that severe chronic deficiencies of most nutrients impaired the immune response and increased susceptibility to mycotoxicosis and some infectious diseases. Severe nutirent deficiencies were particularly deleterious to the immune system when they occurred early life. This is when the primary lymphoid organs and the maturation of the immune system were developed (Cook, 1991). This may occur when the dietary levels were varied over ranges that were marginally below to well above to those required to meet typical dietary recommendations and in chronic poisoining of mycotoxicosis. There was evidence that the high

Table 2 - Effect of dietary Aflatoxin B_1 (AFB_1) baker yeast (BY) and chlorotetracycline (CTC) on 37 days feed intake, body weight, feed efficiency and biochemical parameters meausered in broiler chickens

Parameters	Control (I)	AFB_1 (II)	BY +AFB_1 (III)	CTC+ AFB_1(IV)	BY + CTC+AFB_1 (V)	SEM
Feed intake (g)	3160b	3037a	3313c	3133b	3133 b	19.80
Body weight (g)	1773a	1661a	1860b	1903b	1856 b	34.20
Feed efficiency (g/g)	1.78	1.82	1.78	1.64	1.68	
GOT (I.U./L)	156.3ab	225.6c	182.0b	204.8b	128.6 a	18.50
GPT (I.U./L)	18.3a	44.0c	29.3b	34.6bc	25.6 ab	3.50
ALP (I.U./L)	39.5a	50.0b	41.1a	47.1b	39.4 a	1.60
AFP (I.U./L)	0.07	0.09	0.07	0.06	0.09	0.02

[a,bc] Means within the same line with different superscripts are significantly different (P<.05).
I.U/L: International Unit /Liter.

carcinogenic potential of AFB_1 wass related to a varied response in susceptible species (Chattopadhyay, 1985). AFB_1 in feed rations has been reported to affect livers, spleen, kidneys, bodywight, feed intake, feed efficiency and some biochemical parameters in all species (Smýth, 1984; Hamýlton, 1984). Both GOT and GPT enzymes are indicators of hepatocellular damage (Kubena, 1990), Serum gamma glutamyl transferase activity, which is sensitive indicator of liver dysfunction, indicating liver inflammation, space space-occuying lesions or obstruction of the biliary tract, was significantly increased by feeding diets containing AFB_1 to broilers Kubena (1990) and Bilgiç et al. (1998), studying broiler chicks and had pethecial hemorrhages in liver and kidneys. This was in agreement with results obtained by Çelik et al. (1999). In the present investigation, the data from the results demonstrated that dietary aflatoxin significantly lowers the feed intake and body weight of the birds. Similar results has been reported by Cova et al. (1994). Sell et al. (1998), studying ducks, had similarly reported decreased feed intake and lower body weight. Our results indicated a significant increasing of GOT, GPT and ALP. Increase in these enzyme concentration may result from many kind of degenerations of livers. However, varying amounts of fatty degeneration was detected in liver cells, confirming the observations of others (Lanza, 1980). For parameters monitored and tabulated in Table 1, abnormal values were observed for all enzymes except ALP except control group. Hayes (1978), studying swine, had similary reported elevated GOT and GPT (Garlých, 1973). Same results has been reported by Brown (1965). Data of this research showed that AFB_1 levels as low as 200 ng/g could affect liver enzymes of broiler chicks. Giambrone (1985) showed that the development of acquarid immunity in turkeys and broilers was significantly when given 200 ng/g of pure AFB_1 capsules. According to Wogan (1974), dietary aflatoxin levels 100 ng/g induced liver carcinoma at an incidence greater than 50%, when feeding was continued up to 80 weeks. The interrelationship between the immune system and carcinogenesis has been reported by Sun (1984). These data, as well as previously reported data by Huff & Doerr (1981). No carcinoma was found in AFB_1 treatment (no additives) even at 200 ng/g levels during 5 weeks in this research. Our results indicated the hemorrachagic anemia syndrome caused by AFB_1 was characterized by pethecial and larger hemorrhages into the musculature and internal organs in treatment 2 and 4 of animals. This is in agreement with results obtained Muller (1970). Lipids in liver increased in broiler chicks fed diet with AFB_1 (Smýth, 1970). Similar results were found in this research in treatments 1 and 2. Aflatoxin in feed rations has been reported to affect body weight by Doerr (1983). Our data show similar results in this research. This is in agreement with results obtained by Sahoo (1993) and Adav (1997) & Sell (1998). Miller (1984) has been reported that chickens suffering from aflatoxicosis have been shown to be hypoproteinemic. The use of a protein-sparing antibiotic that also enchances intestinal absorption of essential nutrienst, in this research was found to improve feed conversion in diets containing antibiotic and baker yeast.

The findings of this research suggested that baker yeast (2, 0%) could partly counteract some of the toxic effects of AFB_1 in growing chicks. In conclusion, further investigations may be necessary in long run and other organic-inorganic compounds against the effects of aflatoxins. The use of viable yeast cultures in poultry diets has given promising results and research has taken one step further by identifying the yeast cell wall as the active component which aids in counteracting mycotoxicosis. Mannanoligosaccharides provide new insights into counteracting several pathogens and toxins besides their major impact on modifying the immune response.

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