

Evaluation of Probiotic to Ameliorate the Toxicity of Aflatoxin in Broiler Chickens

Ali Aghwider², Fadwa Ben Taher³, Abdurzag Kerban¹ and Mujahed Bushwreb^{1*}

¹Department of Physiology, Biochemistry and Nutrition, Faculty Veterinary Medicine. University of Tripoli, Tripoli - Libya P. O. Box 13662 Tripoli – Libya

²Faculty of Agriculture and Veterinary Medicine, University of Azytuna, Tarhuna- Libya

³Faculty of Education, University of Tripoli

Received 29 December 2014/ Accepted 26 February 2015

Abstract

The effects of probiotic (*Bacillus toyoi*, 1×10^9 cfu/kg) on toxicity of aflatoxin B1 were evaluated using a total of 420 day-old sexed commercial broiler chicks which were divided randomly into 7 groups each of 3 replicates of 20 chicks. The treatments include the control diet uncontaminated with AFB1, three dietary treatments contaminated with AFB1 (200, 400 and 800 mcg/kg) and three treatments diet contaminated with AFB1 plus Probiotic (1000 g/ton) were used from 0-6 weeks of age. This study revealed a reduction in feed intake; weight gain and feed efficiency were observed. Significant interaction ($P \leq 0.05$) between AFB1 and probiotic were observed for their additive effects on body weight, feed intake, feed efficiency, apparent digestibility of protein, fat, fibre and nitrogen free extract (NFE). Probiotic significantly ($P \leq 0.05$) improved body weight, feed intake and feed efficiency, increased apparent digestibility of protein, fat, fibre and NFE. These results suggest that the addition of probiotic in case of aflatoxicosis may ameliorate and improve the performance as well the retention of nutrients with broiler chickens.

Keywords: Aflatoxin, Aflatoxicosis, Body weight gain, Feed intake, Feed conversion rate

Introduction

Among the known mycotoxins, aflatoxin is the most important to poultry. Aflatoxin B1 is the most potent hepatotoxic and immunosuppressive. It is considered very harmful to human and animal health thus causing significant financial losses to animal industries (Munkvold, 2008; Zhang and Caupert, 2012). It has been reported as well to cause oral lesions and decreased feed intake in broiler chickens. This mycotoxin contaminated feedstuffs when consumed, produce a range of devastating effects on the general well-being and productivity of poultry (Devegowda *et al.* 1998a, Van Leeuwen *et al.* 2005, Egner *et al.* 2003). Feeding AFB1 contaminated diets decreased average daily gain and average daily feed intake linearly and quadratically (Zhang, 2013). Fei Shi *et al.* (2013) investigated the effects of feeding corn contaminated with aflatoxin B1 concluded that AFB1 contaminated diet significantly decreased the body weight gain, feed intake and feed conversion rate.

Practical methods to detoxify mycotoxin contaminated grain on a large scale and in a cost effective manner are currently not available. At present, one of the most promising and practical approaches is the use of adsorbents. Research indicates that a number of adsorbents are capable of adsorbing aflatoxin B₁ and reducing its toxic effects.

A natural product called glucomannan, a cell wall derivative of *Saccharomyces cerevisiae*, has received much attention in minimizing mycotoxins present in the contaminated diets of livestock and poultry (Devegowda *et al.* 1998b; Whitlow *et al.* 2000; Smith *et al.* 2000).

Some dairy strains of lactic acid bacteria were found capable of removing aflatoxin B1 from contaminated liquid media via a rapid process involving the removal of approximately 80% of AFB1 immediately upon contact without further incubation (El-Nezami *et al.* 2000a, 1998b). Furthermore, Audisio *et al.* (2000) claimed that chicks treated with probiotic *Enterococcus faecium* in a preventive way survived to an experimental *Salmonella pullorum* infection. The objective of the present study was to evaluate the efficacy of probiotic (*Bacillus toyoi*, 1×10^9 cfu/kg) as feed additives in aflatoxicosis on performance and nutrients digestibility.

Material and Methods

A total of 420 day-old sexed commercial broiler chicks were divided at random into 7 groups each of 3 replicates of 20 chicks. The test diets treatments consisted of a control diet obtained from a commercial feed mill Al Tahadi (Table 1) and the test diets was prepared by addition of moldy rice. Moldy rice powder was obtained by inoculating known amount of broken rice with *Aspergillus flavus* supplied by Agriculture Research Centre – Tripoli. AF production was carried out in accordance with the method based on that reported by Shotwell *et al.* (1966). Quantified by AFLATEST. Probiotic (1000g/ton) Tyocerin (*Bacillus toyoi*, 1×10^9 cfu/kg) a proprietary product of (Lohmann Animal Health GmbH & Co. KG Heinz-Lohmann-Straße 4 27472 Cuxhaven, Germany), was tested from 0-6 weeks of age. The birds were randomly assigned to the following treatment groups. Control diet without



additives (treatment 1), AFB1 200mcg/Kg (treatment 2), probiotic +AFB 1 200 mcg/Kg (treatment 3), AFB1 400 mcg/Kg (treatment 4), probiotic +AFB1 400 mcg/Kg, (treatment 5), AFB1 800 mcg/Kg (treatment 6), probiotic +AFB1 800 mcg/Kg, (treatment 7), Feed and water were provided for ad libitum Feed intake and faeces output and performance parameters from 0-6 weeks were measured. The data obtained were analysed with SAS, GLM procedure and means were compared with Duncan multiple range test.

Results and Discussion

Aflatoxin (AFB1) individually depressed body weight and feed efficiency (Table 1). The weight gain was lower in chicks fed aflatoxin of 26%, 42% and 44.5% for diets of (200, 400 and 800 mcg/Kg aflatoxine) respectively.

Table 1. Feed composition (Al tahadi Factory, Tripoli)

Item	%
Com	60
Soy Bean	27
Fish Meal	6
Vegetable Oil	2
Mehtionine	0.035
Dicalium Phosphate	2
Salt	1.62
Lime Stone	1
Premix	0.3
Determined Analysis	
Moisture	9.5
Crude Protein	20.57
Ash	9.77
Ether Extract	3.23
Crude Fiber	2.67
Nitrogen Free Extract	50.73
Calcium	1
Phosphorus	0.40

The addition of the probiotic has dramatically improved the weight gain of chicks fed the three levels of aflatoxicosis diets (Table 2). It is well known that yeast culture, and its cell wall extract containing 1,3-1,6 D-glucan and Mannanoligosaccharide are the important natural growth promoters for modern livestock and poultry production (van Leeuwen *et.al.* 2005). Recently, it has been reported that poultry growth is promoted with the increasing doses of probiotic (Protexin) from 0.5 to 1.5 grams per 10 kg feed. Chicken fed probiotics added to aflatoxin diets

consumed significantly more feed than the aflatoxin fed chicks diets. (Table 2). Decreased water and feed intake, weight loss, dullness, are frequency seen in experimental and natural outbreak of aflatoxicosis in broilers (Lesson *et al.* 1995). Decreased feed consumption during mycotoxicoses has been reported (Kubena *et al.* 1997).

Sharlin *et al.* (1981), they stated that Aflatoxicosis in white leghorn males resulted in decreased feed consumption, body weight, while in broiler breeder males reduction in body weight characteristics were observed. The feed: gain ratios were significantly poorer for chicks fed aflatoxin diet as compared to chicks fed probiotics added to all levels of aflatoxin diet, (200 mcd, 400 meg, and 800 mcg aflatoxine). Probiotic supplementation improved body weight, feed intake and feed: gain ratios in the AF fed groups. These data agree with previous results of the protective effects of biological binding agents' compound (Ledoux *et al.* 1999).

The addition of the probiotics to the aflatoxin diets resulted in higher feed: gain ratio was significantly ($P<0.01$) better than non added probiotics diets (Table 2). Feed conversion ratio as affected by probiotics is the subject of controversy. Although, some studies show that probiotics supplementation in feed of chickens improve the feed conversion ratio, some other suggested no such effect on feed conversion ratio (Ahmad 2004). Shareef and Al-Dabbagh (2009) reported that (*Saccharomyces cerevisiae*) supplementation of broilers, was significantly ($P<0.05$), increase the body weight gain, feed consumption and feed conversion efficiency, studying the effect of adding probiotic to aflatoxin diets on the digestibility of protein, lipid, fiber as well the nitrogen free extract are shown in (Table 3). However the probiotic added to (aflatoxin diet 200mcg, 400mcg and 800 mcg aflatoxine), shown a significant ($P<0.01$) improvements in protein, lipid, fiber and nitrogen free extract digestibility in comparison to aflatoxin diet (Table 3). These beneficial effects might be attributed to its growth promotive effect and ability to trap the mycotoxins irreversibly (Devegowda *et al.* 1996). Other authors did not find improvements in performance but found increased protein, calcium and phosphorus retention (Angel *et.al.* 2005). The intestinal bacterial flora of domestic animals has an important role in the digestion and absorption of feed. It participates in the metabolism of dietary nutrients such as carbohydrates,

Table 2. Effect of treatments on weight gain gm, feed intake gm and feed conversion ratio (mean± Se)

Treatments	Weight gain	Feed intake	Feed conversion
Control	1954.3 ± 7.4 a	3141.2 ± 8.5 a	1.51 ± 0.16 ac
AF (200mcg/Kg)	1445.7 ± 4.9 b	2657.3 ± 5.9 b	1.71 ± 0.13 b
AF(200mcg/Kg)+Probiotic	1819.6 ± 8.7 a	2999.9 ± 8.1 a	1.54 ± 0.29 cd
AF(400mcg/Kg)	1135.4 ± 4.4 c	2196.8 ± 4.1 c	1.77 ± 0.1 ab
AF(400mcg/Kg)+Probiotic	1455.8 ± 9.7 b	2465.7 ± 9.6 bc	1.59 ± 0.3 c
AF(800mcg/Kg)	1083.1 ± 4.9 e	2115.9 ± 5.4 d	1.82 ± 0.22 a
AF(800mcg/Kg)+Probiotic	1318.2 ± 8.3 d	2406.9 ± 10.1 bc	1.69 ± 0.32 bc

(a,b,c,d,e,f mean with different superscript are statistically different within the same column $p>0.01$)



Table 3. Effect of treatments on digestibility of crude protein (CP), crude fiber (CF), ether extracts (EE) and nitrogen free extract (NFE). (Mean±Se)

Treatments	CP %	EE %	CF %	NFE %
Control	56.49±4.0 ba	69.83±4.0 a	50.07±4.0 a	50.45±4.3 a
AF (200mcg/Kg)	52.46±5.2 b c	64.25±6.4 b	45.06±4.5 b	42.76±4.1 b
AF(200mcg/Kg)+Probiotic	54.17±4.7 ab	67.58±5.7 ab	47.29±5.1 ab	46.65±4.5 ab
AF(400mcg/Kg)	43.76±4.9 d	60.09±7.1 c	40.59±6.01 c	33.79±5.1 c
AF(400mcg/Kg)+Probiotic	49.67±7.01 c	64.05±6.4 b	44.22±5.7 b	40.66±5.07 b
AF(600mcg/Kg)	36.61±6.4 f	49.24±5.9 e	34.74±4.9 d	21.40±6.01 e
AF(600mcg/Kg)+Probiotic	39.64±5.8 e	55.78±4.8 d	38.47±4.4 c	28.54±5.9 d

(a,b,c,d,e,f mean with different superscript are statistically different within the same column p> 0.01)

proteins, lipids, and minerals and in the synthesis of vitamins. (Nahanshon *et al.* 1994) observed that addition of *Lactobacillus* cultures in maize/soybean or maize / barely / soybean diets stimulated appetite and increased fat, nitrogen, calcium, phosphorus, copper, and manganese retention in layers.

Conclusion

The results indicate that supplementation of probiotic (*Bacillus toyoi*, 1x10⁹ cfu/kg) is beneficial in preventing the individual toxicity of aflatoxin (AFB1) in commercial broilers.

References

- Ahmad I. (2004). Effect of probiotic (Protexin) on the growth of broiler with special reference to the small intestinal crypt cells proliferation. M. Phill Thesis Centre of Biotechnology, Univ. Of Peshawar.
- Angel R, Daloui RA and Derr J. (2005). Performance of broiler chickens fed diets supplemented with a direct fed microbial. *Poultry Sci.* 84: 1222-1231.
- Audisio MC, Oliver G. and Apella MC (2000). Protective effect of *Enterococcus faecium* J96, a potencial probiotic strain on chicks infected with *Salmonella Pullorum*. *J Food Prod.* 63:1333-1337.
- Devegowda G, Arvind BIR. and Morton MG. (1996). *Saccharomyces cerevisiae* and mannan oligosaccharides to counteract aflatoxicosis in broilers. *Proceedings of the Eighth Australian Poultry Science Symposium, Sydney, Australia.* 8: 103-106.
- Devegowda G, Raju MVLN. and Swamy HVLN. (1998a). Mycotoxins: Novel solutions for their counteraction. *Feed stuffs.* 70: 12-15.
- Devegowda, G., Raju, M.V.L.N., Afzali, N. and Swamy, H.V.L.N. (1998b). Mycotoxin picture worldwide: novel solutions for their counteraction. In: *Biotechnology in the Feed Industry, Proceedings of the 14th Annual Symposium (T.P. Lyons and K.A. Jacques eds.), Nottingham University Press.* pp. 241-255.
- Egner PA, Munoz A and Kensler TW. (2003). Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. *Mutat Res.* 523-524, 209-216.
- El-Nezami H, Kankaanpaa P, Salminen S and Abokas j. (1998). Ability of dairy strains of lactic acid bacteria to bind a common foodcarcinogen, aflatoxin B1. *Food chem Toxicol.* 36:321-326.
- El-Nezami H, Mykkanen H, Kankaanpaa P, Salminen S and Ahokas J. (2000). Ability of *Lactobacillus* and *Propionibacterium* strains to remove aflatoxin B 1 from the chicken duodenum. *J Food Prot.* 63: 549-552.
- Fei Shi, Ziaolan Seng, Huagio Tang, Shiming Ahaio, Yang Deng. Ruwen Jin and Yinglun Li (2013). Effect of low levels of Aflatoxin B1 on performance, serum biochemistry, hepatocyte apoptosis and liver histopathological change of cherry valley ducks. *J Anim Vet Adv.* 12: 1126-1130.
- He J, Zhang KY, Chen DW, Ding XM, Feng GD and Ao X. (2013). Effects of maize naturally contaminated with aflatoxin B1 on growth performance, blood profiles and hepatic histopathology in ducks. *Livest Sci.* 152: 192-199.
- Kubena LF, Harvey RB, Buckley SA, Edrington TS. and Rottinghaus GE. (1997). Individual and combined effects of moniliformin present in *fusarium fujikuroi* culture material and aflatoxin in broiler chicks. *Poultry Sci.* 76:265-270.
- Ledoux DR, Rottinghaus GE, Bermudez AJ and Alomo Debolt M. (1999). Efficiency of hydrate sodium calcium aluminasilicate to ameliorate the toxic effect of aflatoxin in broiler chicks. *Poultry Sci.* 78: 204-210.
- Lesson S, Diaz G and Summer JD. (1995). Aflatoxins in poultry metabolic disorder and mycotoxins. Published by University Books. 14, 249-298.
- Nahashon, S., Nakaue, H. and Mirosh, L. (1994). Production variable and nutrient retention in single Comb white laying pullets fed diets supplemented with direct-fed microbials. *Poultry Sci.* 73: 1699-1711.
- Shareef AM. and Al-Dabbagh ASA. (2009). Effect of probiotic (*saccharomyces cerevisiae*) on performance of broiler chicks. *Iraqi J Vet Sci.* 23: 23-29.
- Sharlin J, Howarth B, Thompson F and Wyatt R. (1981). Decreased reproductive potential and reduced feed consumption in mature white leghorn males fed aflatoxin. *Poultry Sci.* 73: 1699- 1711.



- Shotwell OL, Hesseltine CW, Stubblefield RD, Sorenson WG. (1966). Production of aflatoxin on rice. *Appl Microbiol.* 14: 425-428.
- Smith T, Modirsanei M and Macdonald E. (2000). The use of binding agents and amino acids supplements for dietary treatment of Fusarium mycotoxicoses. In: Biotechnology in the Feed Industry. *Proceedings of the 16th Annual Symposium.* (T.P. Lyons and K.A. Jacques, eds), Nottingham University Press, UK. pp 383-390.
- Van Leeuwen P, Verdonk J and Kwakernaak C. (2005). Effects of fructo oligo saccharide (OF) inclusion in diets on performance of broiler chickens. Confidential report 05/I00650 to Orafiti.
- Whitlow LW, Diaz DE, Hopkins BA. and Hagler WM. (2000). Mycotoxins and milk safety: The potential to block transfer to milk. In: Biotechnology in the Feed Industry. *Proceedings of the 16th Annual Symposium.* (T.P. Lyons and K.A. Jacques, eds), Nottingham University Press, UK. pp 391-408.
- Wu F and Munkvold G.P. (2008): Mycotoxins in ethanol co-products: Modeling economic impacts on the livestock industry and management strategies. *J Agric Food Chem.* 56, 3900–3911.
- Zhang Y and Caupert J. (2012): Survey of mycotoxins in U.S. /distiller's dried grains with solubles from 2009 to 2011. *J Agric Food Chem.* 60: 539–543.