Iran. J. Ichthyol. (Special Issue 1, 2022): 166-169

P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

Effect of bread yeast and anti-mycotoxin, bentonite on the qualitative traits of red lying hens

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Abstract
This study was conducted to investigate improving the specific characteristics of egg-laying hens using bentonite and bread yeast to reduce the negative effects of on the qualitative traits of the produced eggs. A total of 76 laying hens, Lohman brown at the age of 40 weeks, distributed into five treatments replicated three times that each replication includes five hens. The first treatment was the control, without any additive and the second, third, fourth, and fifth treatments received adding 3mg aflatoxin/kg feed, 3g bentonite, 5g bread yeast, and 3g bentonite and 5g bread yeast, respectively. The 3mg aflatoxin/kg feed resulted in deterioration of the qualitative traits of the eggs such as the egg weight, shell weight, albumen index, yolk index, hue unit, and SWUSA. All other treatments showed significant enhancement in the studied qualitative traits of the eggs except the thickness of the shell. Treatment 5 was the best treatment, at the age of 49-50 weeks, indicating that the used feed additives reduced or prevented negative effects caused by mycotoxin leading to deteriorating the red hens' egg quality.

Keywords: Aflatoxin, Yeast, Bentonite, Eggshell weight.

Citation: Attallah, O.K.; Abdullah, O.M. & Alnori, H.M. 2022. Effect of bread yeast and anti-mycotoxin, bentonite on the qualitative traits of red lying hens. Iranian Journal of Ichthyology 9(Special Issue 1, 2022): 166-169.

Introduction

The eggshell is an important component that protects egg albumen and yolk. About 10% of the produced eggs are exposed to breakage during transportation from the fields to the consumer. Hence, the smaller the quantity of broken or cracked eggs, the greater the economic return. Melissa et al. (2016) pointed out that aflatoxin B1 causes destruction of the ovary in turkey and the unsaturated oils, raising the free radicals, decreasing the percentage of egg production and the egg weight that would reflect negatively on the egg mass and reduce the eggshell. Mycotoxins are among the most important problems in the poultry industry in Iraq for their spread and role in causing several metabolic and physiological troubles; furthermore, getting rid of the mycotoxins and discarding them out of the bird's body is considered complex (Gulet at el. 2016). According to the toxin concentration in the feed, aflatoxin poisoning leads to a physiological disturbance in birds that cause a significant decrease in the percentage of egg production (Ru Jia et al. 2016). These symptoms are accompanied by a high disturbance in the activity of the liver enzymes responsible for synthesizing cholesterol, triglycerides, low-density lipoproteins (LDL), and High-density lipoproteins (HDL) participating in yolk substance construction (Prasai et al. 2018).

Studies have trended to active approaches for reducing the harm of mycotoxin in the hens through adding some material, decreasing the mycotoxin effectiveness (Zhang et al. 2020), or attaching to them and discarding them. The bread yeast is among these materials that strongly reduce the mycotoxins in the body by attaching to them (Karimi & Zhandi 2015). Bentonite is one of the anti-mycotoxins used as it adsorbs the toxin in the body or neutralizes mycotoxins, thus inhibiting their actions and avoiding the body absorbing those (Yenice et al. 2015). Based on the background mentioned above, this study was conducted to investigate improving the specific characteristics of egg laying hens using bentonite and bread yeast to reduce the negative effects of aflatoxin.

Materials and methods

The isolate of *Aspergillus flavus* was supplied by the Unit of Shared Diseases, College of Veterinary Medicine, University of Baghdad. Rice was used as media for producing aflatoxin B1 according to Shotwell et al. (1966) modified by West et al. (1973) and AL-Warshan (2006). The aflatoxin levels in the sample extracts were measured using the Enzyme-Linked Immune Sorbent (ELISA). The extraction was performed by taking 5g of ground rice and adding 25ml of ethanol 70%. The mixture was stirred for three minutes. To obtain the extracts, the samples were filtered by filter paper (Whatman No.1). Finally, the aflatoxin B1 value was measured according to the protocol recommended by the supplying company.

Field experiment: The used cage was 1x2 in size, the lighting 16L+8D. The feed contains 2700kc/kg metabolized energy and 15.5% raw protein supplied to the hens as 125g/bird/day using hanging cylindrical feeders capacitating for 5kg (Table 1). Water was supplied to the hens all day using inverted founts of 5-liters capacity. A total of 75 Lohman brown laying hens aged 40 weeks were divided into five treatments, replicated three times, comprising 5 hens. The study included the following treatments: (1) without any additive as control, (2) adding 3mg aflatoxin/kg, (3) adding 3mg aflatoxin/kg + 3g Bentonite/kg, (4) adding 3mg aflatoxin/kg + 2g bread yeast/kg, and (5) adding 3mg aflatoxin/ kg + 3g Bentonite/kg + 2g bread.

Egg qualitative traits: Four eggs were taken from each replicate at the end of the study period to measure the qualitative characteristics. They were preserved in a refrigerator for 24 hours for the egg's contents to remain still, and the thick albumen takes its full gelatin texture to facilitate measuring its height. The qualitative traits of the eggs were measured according to Al-Fayyad et al. (2011) (Table 2).

Statistical analysis: The experiment was conducted as a Completely Randomized Design. Data were collected and analyzed using the SAS software (version 9.1, 2010). The significance of differences between the means was tested according to the multiple-rage Duncan test (Duncan 1955).

Results and Discussion

The treatment T5 had the highest egg weight compared to the other treatments. The T3 and T5 had significantly higher shell thickness, whereas the difference was not significant between T1-T5. The T1 showed a significantly higher yolk weight. The T5 had also a significantly higher albumen index, whereas the T1 had a significantly higher yolk index and the lowest one recorded in T5. Regarding the hue unit, T5 was significantly higher than other treatments, while T3, T4, and T5 had significantly higher SWUSA compared to the others. The difference in the egg weight between T1, T3, and T4 was not significant; however, these treatments were significantly higher than that of T2. For the shell thickness, the results showed no significant differences between the treatments. The results also revealed significantly higher ($P \leq 0.05$) albumen weight in T4 and T5 in comparison to the other treatments. In the yolk weight, the T1 was

Table 1. The percentages of feed used in laying hens.

Ingredients (%)	Productivity feed
Yellow corn	57.6
Wheat	10
Wheat bran	5
Soybean cake (48% protein)	14
Center for Protein *	5.0
Limestone	8.1
(Calcium carbonate)	0.2
Table salt	0.1
Total	100
Calculated chemical composition **	
Energy represented (kilocalories / kg)	2754
Crude protein (%)	15.55
Lysine (%)	0.86
Methionine + cysteine%	0.66
Calcium (%)	3.4
Phosphorus is available%	0.37

*Center animal protein user (Brufemi / Jordanian origin) containing 45% protein 2200 kilocalories / Energy represented. 6% fat, 3.5% crude protein, 6.5% calcium, 3% available phosphorous, 2.75% lysine, 1.8% methionine, 2.3% methionine + cysteine. ** The chemical analysis of the components of the diets was calculated according to what was stated in NRC 1994.

Table 2. The results of treatments on the egg characters in this study.

Transactions adjectives	T1	T2	T3	T4	T5	Average in character	Level of morale
Egg weight	61.46^{b}	50.9^{a}	61.47^{b}	62.44^{b}	65.17^{c}	60.29	0.05
Thickness of the shell	0.43^{a}	0.4^{a}	0.45^{a}	0.44^{a}	0.49^{a}	0.44	N.S
Weight crust%	9.14^{a}	9.07^{a}	9.7^{a}	10.07^{b}	10.37^{b}	9.67	0.05
Whiteness weight%	57.3^{a}	58.08^{a}	61.31^{b}	60.43^{b}	61.5^{b}	59.73	0.05
Yolk weight%	30.28^{b}	27.55^{a}	25.71^{a}	26.07^{a}	25.03^{a}	26.93	0.05
Whiteness guide	43.41^{b}	23.83^{a}	42.62^{b}	45.68^{b}	57.52^{c}	42.61	0.05
Yolk Handbook	0.44^{b}	0.38^{a}	0.41^{b}	0.41^{b}	0.42^{b}	0.41	0.05
Hue unit	79.32^{b}	68.29^{a}	77.59^{b}	80.36^{c}	87.05^{d}	78.53	0.05
SWUSA	77.33^{b}	72.54^{a}	82.04^{c}	82.74^{c}	89.25^{d}	80.78	0.05

significantly higher than the others. The results also showed a significantly higher ($P \leq 0.05$) hue unit of the treatment T4. As shown in Table 2, the T5 was the best treatment in most egg qualitative traits compared to the other treatments, whereas the T2 showed the lowest values of the egg qualitative traits.

The results showed significant deterioration of the qualitative traits in the treatment T2 i.e. using adding 3mg aflatoxin/kg. This effect may be due to the negative effects of aflatoxin on the metabolism and optimum absorption of the nutritional elements, via the intestinal villi, which decreases the weight of the egg and its internal components, thus negative effect on the egg production and egg quality (Abd et al. 2019). The cause may be due to Epoxide resulting from the aflatoxin metabolism within the body where it binds with the protein prevent it from performing its physiological functions as enzymes and hormones that, in turn, have to do with providing the amino acids necessary for albumen construction (Rudyk et al. 2019).

Based on the results, the yeast showed a leading role in binding the mycotoxin molecule and excreting it by reducing or inhibiting its effects on the gastrointestinal tract cells and preserving the intestinal villi, which has been reflected positively on the qualitative traits of the eggs (Atamaleki et al.

2019). The anti-mycotoxin (Bentonite) activity was restricted to not expose to the negative damage caused by aflatoxin. Thus, it contributed to better digestion and absorption of nutrients. The synergistic action between yeast and bentonite played an essential role in preserving the membranes lining the intestinal tract that may lead to maintaining the membranes lining intestinal cells and protecting them from the negative effects caused by mycotoxins (Atamaleki et al. 2019). The synergistic mixture of the yeast and bentonite helped to improve the digestion and nutritional element absorption for forming the albumen and yolk as well as the eggshell indicating that it can be used in the feed to enhance these traits and decreased the harmful effect of mycotoxin i.e. The synergistic effect the yeast and bentonite showed reducing of the aflatoxin effects on the qualitative traits of the egg encourage its use in the feed to reduce the negative effect of mycotoxins.

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