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Reserch Article

The Effect of Addition of Activated Bentonite to Aflatoxin Contaminated Feed on Meat Quality of Broiler Chickens

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Abstract

This study was carried out to determine the effects of natural and activated bentonite addition to aflatoxin contaminated feed on meat quality of broiler chickens. In the study, 216 one-day-old broiler chicks (Ross-308) used in this study. Bentonite provided from special mine in Eskişehir-Seyitgazi in Turkiye. The experimental groups consisted of 6 treatment groups with 6 replicates with 6 animals in each group [no AFB1+bentonite, no AFB1+0.4% natural bentonite, no AFB1+0.4% active bentonite, 0.05 ppm AFB1+bentonite, 0.05 ppm AFB1+0.4% natural bentonite, 0.05 ppm AFB1+0.4% active bentonite]. Feed and water were given ad-libitum and the study was continued for 42 days. At the end of the study, meat quality characteristics (breast, thigh and liver pH, L*, a*, b*) of a total of 60 animals (10 animals from each treatment group) were determined. As a result, it was determined that AFB1 decreased the thigh and liver pH levels of broiler chickens ($P<0.001$), but did not affect the breast pH level ($P>0.05$); the addition of active and natural bentonite did not affect the breast and thigh pH levels ($P>0.05$). AFB1 decreased breast a* level ($P<0.05$), increased liver L* and b* levels ($P<0.05$); active and natural bentonite supplementation did not affect breast meat, thigh meat and liver colour values ($P>0.05$). In this study, the effects of bentonite at 0.4% levels were evaluated and it is thought that a comprehensive study at different value levels in the future will give more useful results to the scientific community in this field.

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1. Introduction

The poultry sector has an important position in the world in terms of white meat production, which is a very valuable and economic protein source, and the employment and economic values it creates. The most important input of the sector is feed raw materials. All of the raw material needs of the feed industry cannot be met by domestic resources in the world and are imported. Low quality feed raw materials are used in rations, sometimes for economic reasons and sometimes out of necessity, and economic losses occur (Korkmaz and Gündüz, 2018; Azeem et al., 2019). Storage of poultry feeds under inappropriate conditions causes the growth and proliferation of moulds and microorganisms. This leads to the formation of mycotoxins in feeds. Mycotoxins, which are metabolic products of molds, are toxic compounds that can cause various health and product quality problems in animals (Gruber-Dorninger et al., 2019). Aflatoxins are the most commonly isolated toxic metabolites produced by fungi of the genus *Aspergillus* (Aflatoxin B1) and are the most dangerous mycotoxins for poultry health. Aflatoxins cause decreased growth performance of poultry, increased mortality, loss of quality in meat, decreased egg yield and weight, anorexia (loss of appetite), suppression of the immune system against infections, histopathological damage to the liver, blood clotting disorders, anemia and problems in fat, carbohydrate and protein metabolism (Hernandez-Ramírez et al., 2020). One of the most suitable detoxification methods in terms of effectiveness and ease of application in aflatoxicosis cases is the use of bentonite as a toxin binder with no nutritional value, which protects the health of animals by preventing the absorption of mycotoxins from the digestive system and prevents the transport of toxins to animal products. Bentonite was formed over millions of years as a result of natural geological and biological events such as chemical weathering or alteration of volcanic ash, tuff and lava rich in aluminum and magnesium content (El-Nagar and Sary, 2021). Therefore, it is a substance whose mineral content and quality can vary greatly. Due to these changes in their content, they have different physical, chemical compositions and engineering properties such as high surface areas, electrostatic properties, biogeochemical cycles, etc. (Pasha et al., 2008; Hashemipour et al., 2010; Önal and Özgüven, 2011; Safaeikatouli et al., 2012; Çimen and Dereli, 2014; Gilani et al., 2016; Mishra et al., 2020). Various activation processes are carried out to improve bentonite properties. These processes increase the cation exchange capacity of bentonites, which is the main factor in toxin binding, as well as improving other physical and chemical properties (Kılınç et al., 2022). It has been determined that it has superior physical and chemical properties (Table 1) compared to bentonites used in other studies (Önal et al., 2003; Pasha et al., 2008; Hashemipour et al., 2010; Çimen and Dereli, 2014; Safaeikatouli et al., 2012) with 70% montmorillonite content extracted in Eskişehir Seyitgazi region, which constitutes the main material of the study. In addition, it has been stated that the addition of bentonite to AFB1 contaminated broiler feeds can reduce aflatoxicosis cases and harmful effects in the organism with bentonite (Mgbeahuruike et al., 2018; Rafiu et al., 2019; Saleemi et al., 2020). In broiler breeding, besides developmental performance, meat quality characteristics are also important (Banaszak et al. 2021). Aluminosilicates are used to improve meat quality in broiler diets. Indeed, Hashemi et al. (2014) stated that the addition of zeolite to the diet improved the meat quality of broiler chickens. Banaszak et al. (2020) reported that aluminosilicates increased muscle content and water absorption capacity. It has been proven in many studies that the addition of bentonite to poultry diets improves meat quality (Bouderoua et al. 2016; Attar et al. 2019). In this study, the effects of natural bentonite and activated bentonite addition to aflatoxin contaminated diets on meat quality in broiler chickens were evaluated.

2. Materials and Methods

2.1. Animals and housing

In the study, 216 one-day-old male chicks (Ross 308) were used. Before the chicks were brought to the poultry house, wood shavings were spread on the floor of the existing pens (100*150 cm). The chicks were taken to the broiler house, weighed and distributed to the pens by making sure that the body weight was similar. The temperature inside the poultry house was kept at 32-35^{o}C in the first week, and gradually decreased to 25^{o}C in the second week and then to 20^{o}C. Daylight during the day and fluorescent lamps at night were used for lighting the poultry house. In the poultry house, 23 hours of light and 1 hour of darkness were applied with daylight. Feed and drinking water were given ad-libitum (free).

2.2. Feeding groups

The bentonite used in the experiment was obtained as natural and active bentonite from the Catchem Madencilik AŞ company located in Seyitgazi district of Eskişehir province. The chemical structure of bentonite used in the study is given in Table 1. The chemical analysis of natural bentonite was carried out by Catchem Madencilik AŞ. Aflatoxin B1 (AFB1) (Sigma, No: A6636; Aflatoxin B1 from *Aspergillus flavus*) was used in the study. AFB1 in pure form was dissolved in chloroform solution (1 mg/10 mL) and applied as a spray to the weekly compound feed to be given to the animals.

Table 1. Chemical structure of natural bentonite

	Contents, %							
	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	TiO_2
Natural Bentonite	72.05	20.05	1.05	1.92	2.45	1.61	0.75	0.12
Active Bentonite	Activated by treatment of natural bentonite with 3% soda.							

Standard compound feeds produced by Samsun Yem Sanayii AŞ, in granular form and whose raw material and nutrient contents are given in Table 2, were used as compound feed material in the feeding of broiler chickens used in the study. In the study, a total of 216 day-old male broiler chicks were divided into 6 treatment groups (G-power, Version 3.1.9.4) with 6 replicates and 6 animals in each sub-replicate (G-power, Version 3.1.9.4) in 6 treatment groups [without AFB1 addition+bentonite addition, without AFB1 addition+0. 4% natural bentonite, no AFB1 addition + 0.4% active bentonite, 0.05 ppm AFB1 + no bentonite addition, 0.05 ppm AFB1 + 0.4% natural bentonite, 0.05 ppm AFB1 + 0.4% active bentonite] were randomly distributed.

Table 2. Components and nutrient content of compound feed given to broiler chickens

Raw Materials (%)	Broiler Starter Feed (0-11 day)	Broiler Growth Feed (11-21 day)	Broiler Development Feed (21-35 day)	Broiler Finishing Feed (35-42 day)
Corn extras	34.00	36.00	36.00	10.00
Soya bean meal (%46)	30.60	15.70	6.00	13.78
Corn	13.90	12.20	15.30	56.60
Full-fat soya	10.70	11.50	17.10	5.70
Corn germ	4.00	6.00	6.00	-
Sunflower seed meal (%36)	3.00	6.00	7.00	6.00
Meat-bone meal	-	8.00	8.00	6.69
Wheat	-	3.00	3.00	-
Dicalcium phosphate (%17)	1.43	-	-	-
Marble powder	0.95	0.39	0.40	-
Salt	0.27	0.22	0.23	0.20
DL-Methionine (%88)	-	-	-	0.23
Liquid Methionine	0.40	0.33	0.30	-
L-Lysine (%79)	0.20	0.15	0.15	-
LysineSulphate	-	-	-	0.46
Threonine	0.13	0.11	0.12	0.09
Vitamin-mineral mix[1]	0.25	0.25	0.25	0.25
Sodium sulphate	0.12	0.10	0.10	-
Anticoccidial	0.05	0.05	0.05	-
Total	100	100	100	100
Chemical Analyses				
Dry matter, %	89.90	89.70	89.70	89.60
Crude protein, %	24.30	21.90	20.50	20.00
Crude oil,%	6,10	7.40	7.40	8.20
Crude cellulose,%	3.00	3.00	3.70	3.40
Raw ash,%	6.10	5.80	5.40	5.60
Starch, %	34,70	37.00	38.00	37.10
Calculated Values				
Metabolic energy,kcal/kg	2950	3015	3100	3150
Lysine,%	1.40	1.20	1.08	1.13
Methionine,%	0.70	0.63	0.56	0.54
Methionine+Cystine,%	1.07	1.04	0.97	0.90
Threonine,%	1.00	0.92	0.87	0.78
Tryptophan,%	0.30	0.26	0.23	0.21
Calcium,%	0.88	1.23	1.23	0.85
Total phosphorus,%	0.71	0.82	0.81	0.71
Usable phosphorus,%	0.46	0.57	0.56	0.48
Sodium,%	0.19	0.23	0.23	0.17

[1]per kg of mixture 12 000 IU retinol, 3 000 UI cholecalciferol, 60 mg α-tocopherol, 4 mg menadione, 3 mg thiamine, 10 mg riboflavin, 50 mg niacin, 14 mg Ca-D-pantothenate, 4 mg pyridoxine, 0.030 mg cyanocobalamin, 0.25 mg biotin, 2 mg folic acid, 250 mg choline chloride, 15 mg Cu, 50 mg Fe, 120 mg Mn, 1.5 mg I, 0.3 mg Se, 100 mg Zn, 0.1 mg Co.

2.3. Determination of meat quality values

After slaughter, pH, L*, a*, b*, a*, b* values were measured in liver, breast and thigh meat samples taken from the animals (one from each replicate). Each measurement was carried out in 3 parallels.

2.3.1. pH

After slaughter, the pH values of liver, breast and thigh meat were determined by calculating the average of the values taken with an electronic pH meter (Testo 205) from 3 different points. The electrode immersed in the meat was kept until the value on the screen of the pH metre was fixed and the measurement process was carried out by reading this fixed value (Cao et al., 2012).

2.3.2. Colour

Liver, breast and thigh meat colour measurements were made with Minolta CR 300 Chroma Meter (Minolta Camera Co., Osaka, Japan). Colour values were determined by averaging the measurements made from 3 different parts of the meat samples from which the skin was removed as stated in (Cao et al., 2012). CIE standards (L* = brightness, a* = red colour intensity and b* = yellow colour intensity) were applied in the analysis (CIE, 1986).

2.4. Statistical analyses

The data of the study were analysed (two-way analysis of variance) using the general linear model procedure of the SPSS package (SPSS for windows, Release 21.0) in a 2 (Aflatoxin B1, AFB1) × 3 (Bentonite, BNT) factorial experimental design. Treatment means were determined by Duncan's multiple comparison test and were considered significant at $p < 0.05$. Results are presented as sample size (n), standard error of the mean.

3. Results and Discussion

Meat quality in the food industry is influenced by many factors. Factors such as nutrition, breeding conditions, genetic structure of the animal, stress management, hygiene, colour and pH level determine the consumer's perception of meat quality. The pH level and colour of meat indirectly affect the quality of meat products offered to consumers such as taste, texture, appearance and texture. While the pH level of meat determines the freshness and durability of meat, colour affects the quality perception of the consumer (Şireli, 2018). It is reported that meat pH is related to the biochemical state of the muscle at the time of slaughter and the development of rigor mortis following slaughter and that there is a significant correlation between meat colour and pH (Fletcher, 1999). The higher the pH of the meat, the darker the colour and the lower the pH, the lighter the colour (Allen et al., 1997).

The effects of the treatments on the pH and colour values of breast meat, thigh meat and liver of broiler chickens are given in Table 3. According to the data obtained, it was determined that AFB1 decreased the pH level of thigh meat and liver of broiler chickens, but did not affect the pH level of breast meat. In addition, it was determined that the addition of natural bentonite to broiler diets increased the liver pH level compared to the control group, while the addition of active bentonite decreased it. In addition, it was determined that the addition of active and natural bentonite to broiler diets did not affect the pH level of breast and thigh meat. In addition, it was determined that the addition of active bentonite to broiler mixed diets decreased liver pH compared to natural bentonite. In terms of breast and thigh meat pH, AFB1×Bentonite interaction was found to be significant. The data obtained from the study are similar to the findings that AFB1 decreased rump pH (Shabani et al., 2016) and liver pH, but had no effect on brisket pH (Ma et al., 2024). In addition, reports that the addition of bentonite to mixed feed increased liver pH, but did not affect breast (Hashemi et al., 2014; Banaszak et al., 2020; Banaszak et al., 2021; Gümüş, 2023) and rump pH (Safaei et al., 2016; Shabani et al., 2016) support the results of the present study. Fletcher (1999) reported that there were differences between chicken breast meats in terms of meat colour in commercial slaughterhouses and that there was a close relationship between meat colour and pH. High muscle pH after slaughter causes poultry meat to be classified as dark, tough and dry (DFD) and shortens the shelf life of these meats. On the other hand, low pH levels in 24-hour pH measurement of meats cause the production of meats with lower water holding capacity and colour intensity, but longer shelf life (Şireli, 2018; Fletcher, 1999). In addition, high pH level and microbial count are a sign of meat spoilage. Low meat pH is one of the main factors for a longer shelf life (Yang et al., 2020). When Table 3 is evaluated in general, it can be concluded that bentonite, which is a strong adsorbent, also adsorbed water and increased water consumption compared to aflatoxin rations and increased water consumption may have shifted the intestinal pH to alkaline. pH has a complex effect on meat colour. One of these is that iron-related reactions are pH dependent. Also, muscle pH affects the water holding capacity of proteins. Therefore, it directly affects the physical structure and light transmittance of meat. In addition, pH affects the enzymatic activity of the mitochondrial system by changing the oxygen availability of iron (Fletcher, 2002).

Table 3. Breast, thigh and liver pH and colour parameters of broiler chickens fed diets with or without aflatoxin B1 (AF), natural (NB) and active (AB) bentonite (BNT) additions

		Breast				Thigh				Liver			
		pH	L*	a*	b*	pH	L*	a*	b*	pH	L*	a*	b*
Factors													
AF													
-		5.757	62.20	2.610	6.226	6.140	62.56	3.959	4.756	5.872	41.32	16.70	11.33
+		5.733	63.08	1.800	6.635	5.958	63.37	3.538	5.615	5.747	45.74	16.52	13.17
BNT													
K		5.738	63.04	1.881	6.502	6.011	63.37	3.602	5.336	5.809[b]	43.57	16.77	12.30
NB		5.779	62.09	2.281	6.343	6.091	62.40	3.554	5.373	5.888[a]	42.87	16.30	12.56
AB		5.719	62.79	2.454	6.447	6.045	63.12	4.088	4.847	5.731[c]	44.15	16.75	11.89
AF × BNT													
	C	5.713[ab]	62.03	2.316	6.109	6.054[b]	62.13	4.175	4.736	5.857	41.42	16.52	10.75
-	**NB**	5.776[a]	61.86	2.082	6.027	6.207[a]	62.57	3.630	4.927	5.998	39.80	16.89	11.54
	AB	5.781[a]	62.70	2.713	6.542	6.159[a]	62.97	4.071	4.604	5.760	42.74	16.69	11.71
	C	5.762[a]	64.04	1.446	6.895	5.969[bc]	64.60	3.029	5.936	5.761	45.72	17.02	13.86
+	**NB**	5.781[a]	62.32	1.759	6.659	5.974[bc]	62.23	3.478	5.820	5.778	45.95	15.71	13.59
	AB	5.656[b]	62.88	2.195	6.352	5.932[c]	63.26	4.106	5.090	5.702	45.55	16.82	12.07
OSH		0.014	0.353	0.184	0,174	0,018	0.264	0.132	0,191	0.018	1.376	0.638	0.911
Main Effect of Factors (P value)													
AF		0.395	0.220	**0.030**	0.255	**<0.001**	0.116	0.102	**0.025**	**<0.001**	**<0.001**	0.724	**0.017**
BNT		0.202	0.535	0.422	0.934	0.060	0.280	0.171	0.445	**<0.001**	0.653	0.707	0.759
AF × BNT		**0.034**	0.530	0.835	0.489	**0.044**	0.066	0.132	0.738	0.069	0.480	0.388	0.324

[a,b,c:] Differences between means with different letters in the same column are significant (P<0.05, P<0.01, P<0.001), P: Significance Level, OSH: Standard Error of the Mean, AFB1: Aflatoxin B1, BNT: Bentonite, K: Control Group, DB: Natural Bentonite, AB: Active Bentonite, L*: Brightness, a*: Redness, b*: Yellowness, +: Present, -: Absent.

It was determined that AFB1 decreased breast a* level, increased thigh b*, liver L* and b* levels and did not affect breast L*. In addition, it was determined that the addition of active and natural bentonite to broiler diets did not affect breast, thigh and liver L*, a*, b* values. In addition, no AFB1×Bentonite interaction was observed in terms of breast, thigh and liver colour values (L*, a*, b*). The findings of the study support the studies that AFB1 decreased breast a* (Ma et al., 2024); increased rump b* (Solis-Cruz et al., 2019); but did not affect breast L* (Ma et al., 2024), breast b* (Armanini et al., 2021), breast L* (Armanini et al., 2021), rump L* (Shabani et al., 2016), rump a* values (Shabani et al., 2016). The colour values (L*, a*, b*) findings of the study are in accordance with the findings that the addition of bentonite to compound feeds has no effect on the colour characteristics of liver, breast and thigh meat of animals (Hashemi et al., 2014; Safaei et al., 2016; Banaszak et al., 2021; Gümüş, 2023). Petracci et al. (2004) determined the colour of broiler breast meat and classified the groups as darker than normal (L* < 50), normal (50 < L* < 56) or lighter than normal (L* < 56). L* value limits were determined similarly to pH and water holding capacity levels. Dark coloured meats (L* <50) were found to have higher pH and cooking level, while pale coloured meats (L* <56) were found to have low pH and low water holding capacity. Van Laack et al. (2000), in an experiment investigating the characteristics of pale broiler breast meat, reported that pale coloured breast meat had low pH (5.7), high L* value (60.0) and high water loss (1.34%). In another study on bright coloured meats, L* values were classified as bright if L* > 53, normal if L* values were between 46-53 and dark if L* < 46 (Bianchi et al., 2006). Zhuang and Savage (2009) classified L* values in chicken meat as bright if L* > 60, medium if L* value is 55-59 and dark if L* < 55. In the present study, L* value of breast and thigh meat was above 61.86 in all groups. B* (yellowness) values of breast and thigh meat varied between 4.604-6.027. When the data of the present study are analysed, it can be evaluated as bright according to the classification of Bianch et al. (2006). The differences between the studies can be attributed to the differences in the degree of toxicity selected, physicochemical properties of bentonite, ration composition without additives, dose of mummies, duration of the experiment, environmental factors of the poultry house and rearing conditions (Lyon et al., 2004; Denli et al., 2005; Gümüş, 2023).

Mancini (2013) reported that besides genetics and age, environmental factors such as stress, ration composition, living conditions and toxication etc. also affect the colour of meat. For example, it was observed that the colour of breast meat darkened and redness increased in broiler chickens consuming conjugated linoleic acid. Nitrate in the ration was also found to increase the red colour of chicken breast meat and turkey meat. It was observed that the presence of excess fish meal, rapeseed meal, DL-methionine and choline chloride in the feed resulted in a bitter, unpleasant, stale taste and odour reminiscent of fish odour (Lyon et al., 2004). Muscle pH has been shown to be related to many meat quality characteristics such as tenderness, water holding capacity, cooking loss, juiciness and microbial stability (shelf life). Differences in brisket colour, mainly due to pH, significantly affect the shelf life, odour development, drip loss, water holding capacity, cooking loss and processing quality of brisket (Fletcher, 2002; Saxena et al., 2009; Chang et al., 2023; Li et al., 2023). Differences in meat colour, which is an indicator of spoilage and shelf life, is an important quality criterion in determining consumer preferences (Mancini, 2013; Cao et al., 2021).

Conclusion

As a result, in this study, AFB1 decreased the pH level of the thigh and liver of broiler chickens, but did not affect the pH level of the breast. It was determined that natural bentonite increased liver pH level and active bentonite decreased liver pH level compared to the control. In addition, it was determined that active and natural bentonite did not affect breast and thigh pH levels. It was observed that AFB1 decreased breast a* level and increased liver L* and b* levels in broiler chickens. In addition, it was determined that the addition of active and natural bentonite to broiler diets did not affect the colour values of liver, breast and thigh meat. In this study, the effects of bentonite at 0.4% levels were evaluated and it is thought that a comprehensive study at different value levels in the future will give more useful results to the scientific community in this field.

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