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EFFECT OF ACTIVATED CLINOPTILOLITE IN AFLATOXIN B1 CONTAMINATED LAYING HEN DIETS ON AFLATOXIN B1 RESIDUES AND QUALITY OF EGGS

ABSTRACT: This study was carried out to determine the effect of a high level of aflatoxin B1 in laying hen diets, supplemented with deactivated and activated clinoptilolite, on inner and outer quality and aflatoxin B1 residues in eggs. Two experimental groups were formed and fed high aflatoxin B1 diets (965 ppb) containing deactivated and activated (450 °C for 60 minutes) clinoptilolite (2% of diet) for 49 days. In the experiment, a total of 960 55-week-old Lohmann LSL (white) laying hens were used. Each group had 8 replicates and 480 hens. Egg weight, inner and outer egg quality parameters and egg aflatoxin B1 levels were determined in a total of 90 eggs collected on the 15th, 30th and 49th days of the experiment. Diets containing deactivated or activated clinoptilolite decreased aflatoxin B1 production in laying hen diets after incubation period of 15 days. Activation of clinoptilolite by heat treatment significantly reduced aflatoxin B1 level in eggs ($p<0.05$). In addition, the use of clinoptilolite as an antifungal agent in the presence of high aflatoxin B1level in layer hen diets significantly increased the weight of eggs and significantly reduced the ratio of broken-cracked and dirty eggs ($p<0.05$). Chicken blood albumin, creatinine and calcium levels were higher in hens fed diet containing activated clinoptilolite ($p<0.05$). However, triglyceride and VLDL levels decreased significantly in the blood of these animals ($p <0.05$). In conclusion, the supplementation of hen diets containing high aflatoxin B1 with activated clinoptilolite improves production performance, egg quality and decreases aflatoxin B1 residue in the egg.

KEYWORDS: aflatoxin B1, clinoptilolite, egg, laying hens

INTRODUCTION

Egg has a great importance in meeting animal protein needs of mankind due to high biological value of its protein. However, it is mandatory that egg is

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produced through safe food production process and feed used in its production is formulized in such a way that it does not create any mycotoxin in egg. In recent years, the use of natural and synthetic zeolites as feed additives in order to prevent feed mycotoxins from passing to egg, utilize adsorbent feature and improve eggshell quality has been brought to agenda. A group of minerals are called zeolites. It has been explored that nine zeolite minerals are located in nature in great amounts. Clinoptilolite (CLP) zeolite has wide inner and outer surface areas for ion-exchange reactions thanks to its cage-like structure. Pores present there amount to about 50% of the volume. They are negatively-charged and have capacity of high ion exchange. However, CLP should be produced and processed using appropriate methods in appropriate conditions in order to utilize these features. CLP is a natural material which can hold water, gases and metal ions in itself in exchangeable situation, does not include hazardous elements, it is resistant for temperatures up to 750 °C and acid-bases (pH 1.5-11) (Baran and Kutay, 1999; Melenova *et al.* 2003).

CLP, a natural zeolite, has been approved by European Union in 1999 as a product which can be used in organic animal production. CLP remains active making ion exchange permanently, beginning from the moment it gets involved in digestive system to the moment it is removed. CLP does not interact with other ration nutrients (vitamins, minerals, etc.) therefore it can be included in complex feed additives safely. CLP can hold moisture and fat to a great extent, therefore it prevents degenerations in feeds caused by moisture during storage and it also prevents mycotoxin formation in feed (Pond *et al*. 1988). It is possible to activate zeolite and increase its porosity and adsorption capacity by acid-washing and heating. In this way, acid-resistant zeolites are advantageous in holding gases such as SO_2, HS and CO_2, as well as drying acidic gases such as Cl (Tsithisvili *et al.* 1992; Ragnarsdottir *et al.* 1996). Silicate structure of zeolite is degenerated by acid treatment. The increase of Si/Al ratio in this way is called dealumination process (Kuhl, 1999). The advantages of dealumination are removal of cations in zeolites, increase in heat-sensitivity, extension of pore size and increase in adsorbent feature of zeolite (Gottardi, 1986). It has been determined that heat treatment performed by anaerobic heating method in zeolite subjected to acid-treatment contributes to the increase in porosity and toxic substances holding. It has been indicated that adsorption capacity of zeolites can also increase because of crystallized spaces left by evaporated water heated at temperature of 350–400 °C. Treatment of zeolite with acids such as HCL, H_3BO_3 and H_3PO_4, and the fact that it is subjected to heat-treatment with various acid concentrations in various periods and temperatures, can make significant changes and improvements in adsorption features (Lee *et al*. 2000; Hwang *et al*. 2002; Rožić *et al*. 2002; Ackley *et al.* 2003; Cheng *et al.* 2005; Campos and Büchler, 2007).

In feeds and foods, there are more than seven mycotoxins that have natural toxicity. They are: aflatoxin, zearalenone, ochratoxin A, citrinine, tirchothecenes, patulin, penicillic acid and ergot alkaloids. Aflatoxins make hepatotoxic, mutagenic and hepatocarcinogenic effect on liver. It has also been determined that 0.5% of aflatoxin taken through feed can pass to egg (Denli *et al*. 2005).

It decreases egg productivity and quality when aflatoxin B1 is included in laying hen diets (Herzallah, 2013). Zeolites added in poultry diets adsorb toxic substances and reduce effect of their accumulation by inhibiting their absorption through digestive tract (Çelebi and Kaya, 2012). Moreover, adding zeolite in diets reduces feed passing velocity through digestive system and causes nutrients to get more exposed to digestive enzymes (Khambualai *et al*. 2009). The objective of this study is to analyze the effect of addition of deactivated CLP of 2% (de-CLP) and activated CLP (a-CLP) periods just after heat-treatment application in laying hen diets on egg quality parameters, aflatoxin B1 levels in eggs and some blood parameters.

MATERIALS AND METHODS

In this study, two experimental groups were formed and laying hens were fed laying hen diets containing de-CLP and a-CLP (Table 1). Experimental diets were supplemented with 2% de-CLP and a-CLP. CLP obtained from Bigadiç was activated by heating at 450 °C for 60 minutes. De-CLP and a-CLP samples were subjected to physical and chemical analyses to determine their characteristics related to adsorption, as well as other chemical contents (Table 1) in MTA laboratories in Ankara. A Total of 960 55-week-old Lohmann LSL (white) hens were divided into two experimental groups and fed diets containing de-CLP and a-CLP during 49 days. Triple laying hen cages were used in the experiment for housing. Hens were organized and placed in cages in such a way that hen house was triple-tier having 4 cage sections in each floor and 5 hens included in each cage section. Thus, 8 replicates including 480 hens for each experimental group using 8 cage blocks were used. Diets containing 965 ppb aflatoxin in the beginning formed the basal diet. After 15 days of basal diet, hens were fed basal diets supplemented with 2% of de-CLP and 2% of a-CLP. Two diets were kept under same storage conditions after adding de-CLP and a-CLP, and the samples were taken from the diets on the 15th day of incubation to analyse mycotoxins.

General growth and production performances were monitored in this research. Egg weight, inner and outer quality parameters of 90 eggs collected from each group on the 15th, 30th and 49th day of the experiment were determined by using egg quality measuring device (DET 6000). Egg yolk color was determined using the device in accordance with Roche colour scale. In the same way, aflatoxin B1 (AFB1) levels in eggs were detected in additional 90 eggs collected on the 15th, 30th and 49th day of the experiment. Blood samples were taken from 24 hens from each group and albumin, ALT, ALP, total bilirubin, creatinine, GGT, LDH, cholesterol, triglycerides, VLDL, and calcium were determined by using autoanalyzer.

Data obtained in the experiment conducted in accordance with randomized blocks experiment plan were analyzed by t-test with the usage of SPSS 15.0 program. Tukey multiple comparison test was used for detection of differences in means of groups.

Table 1. Composition of experimental diet and chemical and physical characteristics of CLP

Contents and analytical composition of basal diet	de-CLP	a-CLP
Corn (g/kg)	545	545
Soybean meal (g/kg)	130	130
Sunflower meal (g/kg)	114	114
Limestone (g/kg)	100	100
Guar meal (g/kg)	20	20
Soybean oil (g/kg)	30	30
Meat-bone meal (g/kg)	25	25
DCP (g/kg)	2.5	2.5
Vitamin-mineral premix (g/kg)	2.5	2.5
Salt (g/kg)	2.7	2.7
DL-Methionine (g/kg)	1.8	1.8
Sodiumbicarbonate (g/kg)	1.6	1.6
L-Lysine (g/kg) Multienzyme+Phytase (g/kg)	1.3	1.3
Clinoptilolite (g/kg)	1.6	1.6
Metabolizable energy (kcal/kg of diet)	20	20
CP (%)	2,800	2,800
Ca, (%)	17.5	17.5
Available P (%)	4.1	4.1
Na, %	0.39	0.39
	0.16	0.16
Chemical/physical composition of CLP		
Oil absorption capacity, ml/100g	38.00	45
Water absorption capacity, ml/100g	18.63	21
Apparent porosity, %	19.10	40
Water absorption, %	17.75	19
Cadmium, mg/kg	1.30	<0.1
Lead, mg/kg	50.90	69.73
Arsenic, mg/kg	58.37	65.76
Mercury, mg/kg	<0.5	<0.5
Cation Exchange Capacity (CEC), meq/g	1.6	2.7
Dioxin (ng/kg)	0.2	0.44

Table 2. Effect of CLP activation on aflatoxin level in diets, performance parameters, egg quality, AFB1 levels in eggs, and some blood parameters

Parameters	de-CLP	a-CLP	P-Value	Sign.
Diet total aflatoxin, ppb (initial)	965±13.2	965±13.2	0.998	NS
Diet total aflatoxin, ppb (15 d)	362±14.4	317±12.2	0.742	NS
Diet AFB1, ppb (15d)	362±14.4	317±12.2	0.742	NS
Egg AFB1 content, ppb	0.246±0.0326	0.202±0.0165	0.0007	p<0.05
Feed intake, g/hen	5,325±17.5	5,066±31.5	0.000	p<0.05
Initial body weight, g	1,845±60	1,839±46	0.934	NS
Final body weight, g	1,899±62	1,888±48	0.969	NS
Liver weight, g/100 g BW	2.473±0.084	2.546±0.11	0.599	NS
Heart weight, g/100 g BW	0.492±0.022	0.486±0.021	0.835	NS
Spleen weight, g/100 g BW	0.123±0.0080	0.137±0.0092	0.266	NS
Egg production ratio, %	85.66±0.38	86.08±0.0032	0.398	NS

Egg weight, g	68.02±0.061	68.23±0.049	0.011	p<0.05
Broken-cracked egg ratio, %	1.995±0.15	1.345±0.11	0.001	p<0.05
Dirty egg ratio, %	3.53±0.32	2.270±0.095	0.000	p<0,05
Haugh Unit, HU	84.85±0.54	83.98±0.55	0.261	NS
Albumin height, mm	7.715±0.085	7.586±0.077	0.263	NS
Egg breakage resistance, STR	4.017±0.098	3.880±0.076	0.275	NS
Shell thickness, mm	0.416±0.004	0.427±0.004	0.064	NS
Shell weight, g	9.428±0.072	9.418±0.069	0.917	NS
Egg yolk colour	12.69±0.088	12.77±0.074	0.473	NS
Albumin, g/dl	2.167±0.062	2.371±0.075	0.041	p<0.05
ALP, U/L	895±75	1,006±115	0.427	NS
ALT, U/L	34.1±9.5	27.4±8.9	0.608	NS
Total bilirubin, mg/dl	0.250±0.026	0.304±0.021	0.110	NS
Creatinine, mg/dl	0.195±0.018	0.292±0.028	0.006	p<0.05
GGT, U/L	42.5±11	32.4±7.3	0.436	NS
LDH, U/L	1,480±254	2,197±285	0.067	NS
Cholesterol, mg/dl	137.3±11	129.6±10	0.607	NS
Triglyceride, mg/dl	1,427±107	1,148±85	0.047	p<0.05
VLDL, mg/dl	285±21	229.5±17	0.047	p<0.05
Calcium, mg/dl	15.73±1.1	17.10±0.74	0.299	NS

RESULTS AND DISCUSSION

The effects of addition of a-CLP, heated at 450 °C for 60 minutes, in laying hen diets containing high levels (965 ppb) of total aflatoxin were examined. Total aflatoxin amount in diets was 362 ppb in the diet containing de-CLP and 317 ppb in the diet containing a-CLP, although at the beginning, diet total aflatoxin level was 965 ppb because of lack of any antifungals in the diet. Effects of treatments on body weight gain, feed intake, feed efficiency and internal organ weights are presented in Table 2. The usage of a-CLP in the diet led to a significant decrease in feed consumption ($p<0.05$). On the other hand, its effect on final live weight, weights of liver, heart and spleen was insignificant. A-CLP significantly ($p<0.05$) reduced aflatoxin B1 levels in eggs. Additionally, a-CLP significantly ($p<0.05$) increased egg weight and reduced rates of broken-cracked and dirty eggs. Haugh unit (HU), albumin height, egg breakage resistance, shell thickness, shell weight and egg yolk colour were not affected by the treatments (Table 2). Albumin, creatinine and calcium levels in blood increased in hens fed diet containing a-CLP ($p<0.05$). On the other hand, triglyceride and VLDL levels in blood of these animals decreased significantly ($p<0.05$).

The decrease in feed intake by usage of a-CLP in the diet makes a remarkable result for production economics. If the level of AFB1 in diet increased, a larger amount of AFB1 would pass to egg and leave residue. In this study, the usage of a-CLP in the diet significantly reduced the AFB1 amount in egg. Similarly, Mizrak *et al*. (2014) reported that no aflatoxin was encountered when

sepiolite was added into laying hen diets in 1.5% and 3% rates. However, it should be taken into consideration that rates of aflatoxins in diets used in these studies were various. Using a-CLP, AFB1 level in egg was significantly reduced ($p<0.05$). Furthermore, it also significantly increased the egg weight and reduced the rate of broken-cracked and dirty egg. HU, albumin height, egg breakage resistance, shell thickness, shell weight and egg yolk color were not affected by dietary treatments. Despite the fact that a statistically significant effect on shell thickness was not observed, the rate of broken-cracked egg decreased significantly due to the usage of a-CLP. Similarly, Bozkurt *et al*. (2001) reported that zeolite usage decreased the rates of broken-cracked egg without affecting shell resistance and egg shell thickness. Çelebi and Kaya (2012) reported that a-CLP significantly increased egg weight and reduced the rate of damaged egg and also improved the egg shell thickness, especially if added during the late period. Mizrak *et al*. (2014) indicated that positive effect of zeolites on eggshell formation and bone development reduced phosphorus utility by forming insoluble aluminium silicate compounds with phosphorus ion in blood plasma of aluminium and silicon ions in its structure. Thus, the absorption of plasma calcium and calcium mobilization from bones were accelerated and the shell quality improved. Likewise, zeolites enhance the usage of vitamin D_3 regulating calcium and phosphorus metabolism and thus have positive effect on shell quality and bone structure. Zeolites perform this effect by binding mycotoxins, significant vitamin D_3 binders, and inhibiting their activity to bind vitamin D_3. It was observed in studies, that there was a decrease in eggshell quality when phosphorus was not included in diet in high amounts (Çelebi and Kaya, 2012). Addition of de-CLP or a-CLP affected egg yield. Similarly, Balevi *et al.* (1998) reported that the addition of zeolite in laying hen diets did not affect egg yield. It was considered that the used dose can be effective as zeolite did not have any effect on egg yield. As a matter of fact, Yalçın *et al*. (1987) reported that the addition of zeolite at 2% rate in laying hen diets did not affect egg yield. However, egg yield was increased by the addition of zeolite at 4% rate. It was observed that the addition of a-CLP in laying hen diets reduced feed intake without affecting egg yield. Similarly, Balevi *et al*. (1998) also reported that addition of zeolite in 2.5% and 3.5% rates in laying hen diets reduced feed intake without affecting egg yield. Despite the findings of Miles *et al*. (1986), reporting that there was a decrease in egg weight by addition of zeolite in 1.5% level in diet for egg weight, it was observed in this study that the egg weight can be also significantly increased, especially by the activation of CLP. On the other hand, Oğuz *et al*. (2017) added perlite, expanded 10–30 times more than its normal volume by heating at temperature of 700–1,000 °C, into laying hen diets in 1.2% and 3% levels and they reported that addition of perlite reduced egg weight. It was considered that zeolite source could have effect if used within this variety. Gezen *et al.* (2004) considered that natural CLP extracted from Manisa region can affect egg weight positively due to its structural difference. It was considered in this study that CLP extracted from Bigadiç region and activated separately put forward this variety. Besides, Machaček *et al*. (2010) reported that the egg weight was increased by the addition of CLP at 2% rate and decreased by its

addition at 4% rate, and thus this situation made scientists conclude that the used dose can also have effect on weight. Among criteria assessed in terms of egg quality, the results found for HU, albumin height, egg breakage resistance, shell thickness, and yolk colour exhibit similarities to the results presented by Öztürk *et al.* (1998), Kralik *et al*. (2006), and Mizrak *et al*. (2014). However, Oğuz *et al*. (2017) found that albumen index was decreased by the addition of perlite. Vogt (1991) reported that the addition of CLP improved yolk color.

Albumin, creatinine and calcium levels were found at higher levels in blood of hens fed diets containing a-CLP ($p<0.05$). On the other hand, triglyceride and VLDL levels decreased significantly in blood of these animals ($p<0.05$). Serum ALP, ALT, total bilirubin, GGT, LDH, and cholesterol levels were not affected by the activation of CLP. Similarly, Mizrak *et al*. (2014) reported the similar results by adding sepiolite. Kralkik *et al.* (2006) added a commercial product called Nanofeed, activated tribomechanically and containing CLP, in laying hen diets, and on the 14th day detected that levels of creatinine, total bilirubin, total protein, globulin and ferritin increased. Denli and Okan (2006) reported that the hydrated sodium calcium aluminosilicate HSCAS in diets containing 80 μg/kg AFB1 inhibited the increase of serum AST in broilers. Increase in AST and ALT in serum is one of well-known effects of aflatoxicosis. It is attributed to protective effect of CLP binding aflatoxin that in this study ALP and ALT levels were not affected.

CONCLUSION

It has been observed that utilization of de-CLP or a-CLP at 2% level in laying hen diets has positive effects on egg quality, yield performance and some blood parameters. It can be said that the increase in cation exchange capacity of CLP by activation also increased the adsorption capacity of CLP for aflatoxin.

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УТИЦАЈ АКТИВИРАНОГ КЛИНОПТИЛОЛИТА НА ОСТАТКЕ АФЛАТОКСИНА Б1 И КВАЛИТЕТ ЈАЈА КАДА ЈЕ ХРАНА КОКА НОСИЉА КОНТАМИНИРАНА АФЛАТОКСИНОМ Б1

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РЕЗИМЕ: Ова студија спроведена је како би се одредио ефекат деактивираног и активираног клиноптилолита код високог нивоа афлатоксина Б1 у храни кока носиља на унутрашњи и спољашњи квалитет јајета, као и на остатке афлатоксина Б1 у јајету. Формиране су две експерименталне групе које су током 49 дана храњене храном с високим нивоом афлатоксина Б1 (965 ppb), а која је садржавала и деактивирани и активирани (грејањем 60 минута на 450 °C) клиноптилолит (2% у храни). У експерименту је испитано укупно 960 јединки 55-онедељних Ломан ЛСЛ (белих) кока носиља. Свака група имала је осам реплика са 480 кокошака. Тежина јајета, параметри унутрашњег и спољашњег квалитета јајета, као и ниво афлатоксина Б1 у јајету утврђени су код укупно 90 јаја сакупљених 15, 30, и 49. дана експеримента. Деактивирани или активирани клиноптилолит смањио је производњу афлатоксина Б1 у храни кока носиља након инкубације од 15 дана. Активација клиноптилолита грејањем значајно је смањила ниво афлатоксина Б1 у јајима ($p<0,05$). Поред тога, употребом клиноптилолита као антифунгалног агенса у присуству високог нивоа афлатоксина Б1 у храни кока носиља значајно

је повећана тежина јајета а значајно смањен однос поломљених и запрљаних јаја ($p<0,05$). Ниво албумина, креатинина и калцијума био је већи код кокошака храњених храном која садржи активиран клиноптилолит ($p<0,05$). Међутим, нивои триглицерида и липопротеина (VLDL) у крви ових животиња значајно су се смањили ($p<0,05$). Може се закључити да суплементација хране кока носиља која садржи висок ниво афлатоксина Б1 активираним клиноптилолитом побољшава производне перформансе, као и квалитет јаја, те смањује остатак афлатоксина Б1 у јајету.

КЉУЧНЕ РЕЧИ: афлатоксин Б1, клиноптилолит, јаје, коке носиље