Kafkas Univ Vet Fak Derg
20 (4): 481-486, 2014
DOI: 10.9775/kvfd.2013.10217

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Efficacy of Dietary Savory Essential Oil on Reducing the Toxicity of Aflatoxin B_1 in Broiler Chicks

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Makale Kodu (Article Code): KVFD-2013-10217

Summary

The aim of this study was to evaluate the capability of savory essential oil in counteracting the deleterious effects of aflatoxin B_1 (AFB_1) on growth performance, serum biochemistry, and humoral immune responses in broiler chickens fed 42 days of age. At a 2^2 factorial arrangement in completely randomized design, 300 day-old broiler chicks were assigned to four treatments with five replicates of 15 birds. Chickens were fed the basal diet up to day 7 of age and then fed the experimental diets. The dietary treatments involved of 0 and 0.5 mg of AFB1/kg with or without 500 mg of savory/kg dry matter. The addition of aflatoxin to diet decreased ($P<0.05$) the weight gain and feed intake and resulted in a poor feed conversion ratio. Birds in the AFB_1 group had lower level of albumin, but higher levels of creatinine and liver enzymes in the serum as compared with the control group. The addition of savory to the contaminated diet diminished ($P<0.05$) the inhibitory effects of dietary AFB_1 on the growth performance and the level of liver enzymes in serum. The addition of aflatoxin to diet caused a decrease and savory supplementation caused an increase in antibody titer against the Newcastle virus, and interaction among the factors was differ ($P<0.05$). The main effects and interaction on antibody titer against influenza virus were not differ ($P>0.05$). The addition of savory reduced the adverse effects of aflatoxin on growth performance and provided slight positive effect on serum biochemistry and humoral immune responses in broilers exposed aflatoxin.

Keywords: *Aflatoxin, Broiler, Savory, Protection*

Broyler Piliçlerde Rasyona Katılan Geyikotu Esansiyel Yağının Aflatoksin B_1 Toksisitesinin Azaltılmasındaki Etkinliği

Özet

Bu çalışma, 42 gün süreyle beslenen broyler piliçlerde yeme katılan geyioktu esansiyel yağının, aflatoksin B_1 (AFB_1)'in büyüme performansı, serum biyokimyası ve hümoral immün yanıtlar üzerine olan zararlı etkilerini yoketme kapasitesinin değerlendirilmesi amacıyla yapıldı. Randomize tasarım ile 2^2 faktöryel düzenlemede, 300 günlük broyler piliçler 15 kanatlı içeren beş tekrarlı dört tedavi grubu olacak şekilde ayarlandı. Piliçler 7. güne kadar temel rasyon ve daha sonra deneysel diyet ile beslendi. Besinsel tedaviler 500 mg kuru madde olarak geyioktu/kg içeren veya içermeyen 0 ve 0.5 mg AFB_1/kg'dan oluştu. Rasyona aflatoksin eklenmesi ağırlık kazancını ve yem tüketimini azaltırken ($P<0.05$) zayıf bir yem dönüşüm oranıyla (poor feed conversion ratio) sonuçlandı. AFB_1 grubundaki kanatlıların kontrol grubuyla kıyaslandığınıda düşük albumin düzeyine, fakat yüksek serum kreatin ve karaciğer enzimleri düzeyine sahip olduğu gözlemlendi. Kontamine rasyona geyioktu ilavesinin besinsel AFB1'in büyüme performansı ve serumdaki karaciğer enzim seviyesi üzerindeki inhibitör etkilerini azalttığı ($P<0.05$) belirlendi. Newcastle virüsüne karşı gelişen antikor titresinin rasyona aflatoksin ilavesi ile azaldığı ve geyioktu ilavesi ile arttığı ve faktörler arası etkileşimin farklı ($P<0.05$) olduğu gözlemlendi. İnfluenza virüsüne karşı gelişen antikor titresi bakımından ana etkilerin ve etkileşimin değişmediği ($P>0.05$) belirlendi. Geyioktu ilavesinin aflatoksinin büyüme performansı üzerine olumsuz etkilerini azalttığı ve aflatoksine maruz kalan broylerlerde serum biyokimyası ve hümoral immun yanıt üzerine hafif bir olumlu etki sağladığı sonucuna varıldı.

Anahtar sözcükler: *Aflatoksin, Broyler, Geyikotu, Koruma*

INTRODUCTION

Aflatoxins are mycotoxins produced by *Aspergillus* species as secondary metabolites [1]. Due to the ubiquitous nature of species *Aspergillus* in the environment, mycotoxin contamination of grains and feed is unavoidable. Toxic

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effects of aflatoxin commonly observed in animals included poor absorption of nutrients which lead to death, reduced tissue integrity, lower growth rates and poor feed conversion, reproductive problems in males and females, increasing sensitivity to extreme temperatures and reduced immune response [2]. Different approaches such as irradiation, mold inhibitors, dietary absorbents, ammoniation and ozone degradation were applied to reduce food and feed contamination by aflatoxins [3,4]. Unfortunately, most of these methods are expensive and only partially effective. Currently, the practical approach has been the addition of feed additives such as essential oils to contaminated feeds to reduce the negative effects of aflatoxin in the body.

Savory *(Satureja khuzistanica)* is an annual, herbaceous aromatic and medicinal plant belonging to the Lamiaceae family with identified essential oils named carvacrol and thymol [5].

Savory is native in western and southern part of Iran and used as an analgesic and antiseptic in folk medicine in the region. The properties of this herb were reported as antimicrobial [6], anti-inflammatory [7], antifungal [8], anti-oxidant [9,10] and anti-hyperlipemia [11]. In the literature, there was no report concerning the effect of dietary essential oil of savory on performance, serum bio-chemistry, and humeral immune responses of broilers exposed to aflatoxin. Therefore, this study was conducted to evaluate the potential of applying essential oil of savory in protecting of aflatoxicosis on performance, serum biochemistry, and humeral immune responses of broilers fed diet containing aflatoxin B_1.

MATERIAL and METHODS

The study was approved by the Ethics Committee of Islamic Azad University, Science and Research Branch of Medical and Veterinary Sciences (approval date: 12.03.2012; no: 1265, AEC 2).

Aflatoxin Production

Aflatoxin was produced from *Aspergillus parasiticus* PTTC 5286 culture through fermentation of rice using the method proposed by Shotwell et al.[12]. The sterile rice was placed in Erlenmeyer flasks and inoculated with 2 ml of the mold aqueous suspension containing 10^6 spores/ml. Cultures were allowed to grow for 7 d at 39°C in incubator. On the seventh day, Erlenmeyer flasks were autoclaved and culture materials were dried for 48 h at 40°C in a forced-air oven and ground to a fine powder. The aflatoxin B_1 levels in rice powder were measured by HPLC method [13]. Of the total aflatoxins content in the rice powder, 84.64% was aflatoxin B_1 and 15.36% was aflatoxin G_1. Aflatoxin B_2 and aflatoxin G_2 were not detected. The milled substrate was added to the basal diet to provide the level of 0.5 mg/kg of aflatoxin.

Animals and Dietary Treatments

A total of 300 day-old broiler chicks (Ross 308) were purchased from a local hatchery. On arrival, the birds were weighed and randomly allocated to one of the four treatments with five replicates of 15 birds based on a 2^2 factorial arrangement in completely randomized design, consisted of two levels of AFB_1 (0 and 0.5 mg/kg of feed) and two levels of savory (0, 500 mg/kg of feed).

Chicks up to day 7 of age are very sensitive to aflatoxins, thus they were fed diet without aflatoxin. Thereafter, until day 42 of age (8-42 days), they were fed the experimental diets. The isonitrogenous and isocaloric diets were formulated to meet the nutrients ratio and requirements of broilers as recommended by Ross [14]. Essential oils from savory were obtained by Clevenger-type water distillation. The major compounds in savory essential oils were identified using gas chromatography in another study [15] and consisted of carvacrol (80.6%), p-cymene (4.8%), myrcene (1.5%), terpinene (2.1%) and terpinene-4-ol (2.1%). The appropriate amount of savory extract for 100 kg of feed was premixed with a carrier of 1 kg in order to mix the relevant treatment diet.

Table 1 lists the basal diet formulated to meet the nutrient requirements of broilers. The chicks were raised on floor pens (150 × 150 × 80 cm) for 6 weeks and had free access to feed and water throughout the entire experimental period. The lighting program consisted of a period of 23 h light and 1 h of darkness in 1 day. The ambient temperature was gradually decreased from 33 to 25°C on day 21 and was then kept constant.

Performance and Organs Weight

The body weight was determined at days 14, 28 and 42 of age. Feed consumption and weight gain were recorded at different periods and feed conversion ratio (FCR) was calculated. At day 42 of age, two birds from each replicate were randomly selected based on the average weight of the group and sacrificed. Carcass yield was calculated by dividing eviscerated weight by live weight. Abdominal fat, liver, pancreas was separated, weighed and calculated as a percentage of live body weight.

Vaccination and Immunological Tests

The commercially available oil-adjuvant injectable emulsion against the Newcastle disease virus (NDV) and avian influenza virus (AIV) were used (H9N2 subtype) for vaccinating broiler chicks, and they were injected subcutaneously with 0.2 ml per chick at day 9 of age. Also, the chicks were orally vaccinated against the Newcastle Disease (Lasota) at 21 d of age. Antibody titers against NDV and AIV were measured as immune responses. At day 28 of age, two male broilers from each pen were randomly selected, and blood samples were taken by puncture of the brachial vein for analysis of antibody titers against

NDV and AIV. Serum antibody titers against NDV and AIV were measured by the hemagglutination inhibition test (HI), and HI antibodies were then converted to log2.

Table 1. *The ingredient and calculated composition of basal starter, grower, and finisher diets*

Tablo 1. *Temel başlangıç, büyütme ve bitiş rasyonlarının içeriği ve hesaplanmış kompozisyonu*

Item	Starter	Grower	Finisher
Ingredient, g/kg			
Corn	537.3	533	561
Soybean meal	400	395	370
Soybean oil	20	35	35
Di calcium phosphate	19.3	17.7	15.6
$CaCO_3$	10.5	8.8	8.5
NaCl	3.5	3	3
Trace mineral premix[a]	2.5	2.5	2.5
Vitamin premix[b]	2.5	2.5	2.5
DL-methionine	3.1	2	1.4
L-lysine	1.3	-	-
Savory	-	0.5	0.5
Analyzed Calculation			
Metabolizable energy (kcal/kg)	2870	2980	3000
Crude protein (g/kg)	221	220	210
Calcium (g/kg)	8.6	7.5	7
Available phosphorus (g/kg)	4.9	4.4	4.1
Methionine+cysteine (g/kg)	1.1	8.9	8
Lysine (g/kg)	13.3	11.9	11.3
Threonine (g/kg)	8.3	8.3	6.3
Tryptophan (g/kg)	3.2	3.2	3

[a] Provided the following per kg of diet: Mg, 56 mg; Fe, 20 mg; Cu, 10 mg; Zn, 50 mg; Co, 125 mg; I, 0.8 mg; [b] Provided the following per kg of diet: vit A, 10,000 IU; vit D_3, 2000 IU; vit E, 5 IU; vit K, 2 mg; riboflavin, 4.20 mg; vit B_{12}, 0.01 mg; pantothenic acid, 5 mg; nicotinic acid, 20 mg; folic acid, 0.5 mg; choline, 3 mg

Serum Biochemical Parameters

After 12 h fasting, blood samples were collected in non-heparinised tube at day 42 of age from 8 birds in each treatment by puncturing the brachial vein and the blood was centrifuged to obtain serum. Serum samples were analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), albumin, total protein, triglyceride, cholesterol and creatinine were determined using an automated analyzer (Technicon RA-1000, Tarrytown, USA) based on the commercial kit protocol (Pars Azmoon Company, Tehran, Iran).

Statistical Analysis

All data were subjected to ANOVA using the General Linear Model procedure of SAS software (SAS Inst. Inc., Cary, NC). The mean differences among different treatments were separated by Duncan's multiple range tests. A level of $P<0.05$ was used as the criterion for statistical significance.

RESULTS

Performance of Broiler Chickens

The effects of treatments on growth performance are shown in *Table 2*. During the total period, a significant interaction was observed between AFB_1 and savory for body weight and feed intake ($P<0.05$), but in terms of FCR, no significant difference was observed. The birds fed diets containing aflatoxin compared with the control group had lower body weight gain and feed intake. Chickens fed diets containing aflatoxin had lower FCR than the control group, but the difference was not significant. The inclusion of savory (500 mg/kg) to the diet of birds fed diets containing aflatoxin affected these factors and leading to a reduction in the negative effects of aflatoxin. During the total period, the feed intake, body weight gain and FCR in the birds

Table 2. *Effect of savory on growth performance for broiler chicks fed diet containing 0.5 mg aflatoxin/kg at 1 to 42 d of age*

Tablo 2. *1. - 42. günlerde 0.5 mg aflatoksin/kg içeren rasyon ile beslenen broyler piliçlerde geyikotunun büyüme performansı üzerine etkisi*

Treatments	Body Weight Gain (g)				Feed Intake (g)				Feed Conversion Ratio			
	0-14 day	14-28 day	28-42 day	0-42 day	0-14 day	14-28 day	28-42 day	0-42 day	0-14 day	14-28 day	28-42 day	0-42 day
Control	288[a]	857[a]	1030[ab]	2176[b]	436[a]	1506[a]	2237[a]	4157[a]	1.51	1.75[b]	2.19	1.91
AFB1	247[d]	652[b]	859[c]	1753[d]	379[c]	1231[c]	1869[b]	3485[c]	1.54	1.91[a]	2.19	1.98
Savory	282[b]	883[a]	1108[a]	2266[a]	414[b]	1482[ab]	2210[a]	4085[ab]	1.47	1.68[b]	1.99	1.80
AFB1+ Savory	272[c]	825[a]	980[b]	2089[c]	404[b]	1392[b]	2113[a]	3943[b]	1.49	1.69[b]	2.15	1.89
SEM	2.9	18.2	23.3	37.3	4.2	25.2	38.8	54.2	0.011	0.024	0.032	0.016
Probability												
AFB1	**	**	**	**	**	**	**	**	NS	*	NS	**
Savory	**	**	*	**	NS	NS	NS	**	NS	**	*	**
AFB1× Savory	**	**	NS	**	**	*	*	**	NS	*	NS	NS

*[a,b,c,d] Values in the same column not sharing a common superscript differ ($P<0.05$); * $P<0.05$; ** $P<0.0$*

fed savory alone were not differ (P>0.05) as compared with the control group.

Serum Biochemical Parameters

The effects of diets containing aflatoxins and savory on blood biochemical parameters of broilers are shown in *Table 3*. At day 42 of age, a significant interaction was observed between AFB_1 and savory on serum glucose, creatinine, cholesterol, albumin, ALT and ALP. However, there was no significant difference for serum true protein, triglyceride, AST and GGT among treatments (P>0.05). However, feeding the diet containing aflatoxin to broilers decreased the levels of glucose, cholesterol and albumin, and increased creatinine, ALT, and ALP as compared with the control group. The addition of savory (500 mg/kg) to the diet containing aflatoxin ameliorated the adverse effect of aflatoxin on the serum levels of glucose and ALT (P<0.05) as compared with the group fed diet containing aflatoxin without savory.

Relative Organ Weights

The relative weights of some organs are shown in *Table 4*. The main effect of aflatoxin was significantly difference, but the addition of savory to diet of broilers had no effect on the relative weight of liver and spleen. A significant interaction between savory and AFB_1 was observed in the relative weights of liver and spleen (P<0.05). The highest relative weights of liver and spleen were related to the birds fed diet containing aflatoxin. The addition of savory (500 mg/kg) to the diet containing aflatoxin decreased the relative weights of spleen, but had no significant effect on the relative weights of liver. The main effects and interaction on the relative weights of abdominal fat and pancreas were not significantly difference.

Immune Responses

The effects of experimental diets on antibody titers against NDV and AIV at day 28 of age are presented in *Table 5*. A significant interaction between savory and AFB_1 was observed for antibody titers against NDV. Antibody titers against NDV were different among treatments (P<0.05). The lowest antibody titer against NDV was found in the birds fed diet containing aflatoxin and the highest was observed in the birds fed savory alone. The addition of savory to diet containing aflatoxin significantly increased antibody titers against NDV. In the case of antibody titers against AIV, there was no difference among treatments (P>0.05).

DISCUSSION

In this study, the efficacy of savory for protection from the harmful effects of aflatoxin on the performance, health and immunity of broilers was investigated. The results indicated that ingestion of aflatoxins resulted in a significant decrease in feed intake and consequently the

Table 4. *Effect of savory on relative organ weights for broiler chicks fed diet containing 0.5 mg aflatoxin/kg at day 42 of age[1]*

Tablo 4. *42. günde 0.5 mg aflatoksin / kg içeren rasyon ile beslenen broyler piliçlerde geyikotunun göreceli organ ağırlıkları üzerine etkisi[1]*

Treatments	Abdominal Fat Weight (%BW[2])	Liver Weight (%BW)	Spleen Weight (%BW)	Pancreas Weight (%BW)
Control	1.41	2.10^{b}	0.11^{b}	0.25
AFB1	1.31	2.52^{a}	0.14^{a}	0.27
Savory	1.21	2.41^{a}	0.11^{b}	0.26
AFB1+ Savory	1.40	2.42^{a}	0.13^{ab}	0.26
SEM	0.062	0.074	0.011	0.013
Probability				
AFB1	NS	*	*	NS
Savory	NS	NS	NS	NS
AFB1× Savory	NS	*	*	NS

*^{a,b} Means within a column without a common superscript differ statistically (P<0.05); [1] Results are reported as means for 6 broilers each; [2] Body weight; * P<0.05; ** P<0.01*

Table 3. *Effect of savory on biochemical parameters for broiler chicks fed diet containing 0.5 mg aflatoxin/kg at day 42 of age[1]*

Tablo 3. *42. günde 0.5 mg aflatoksin / kg içeren rasyon ile beslenen broyler piliçlerde geyikotunun biyokimyasal parametreler üzerine etkisi[1]*

Treatments	Glucose (mg/dL)	Creatinine (µmol/L)	CHOL (mg/dL)	TRG (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/dL)	Albumin (g/dL)	GGT (U/l)
Control	243^{a}	0.32^{ab}	135^{a}	61.8	349	4.01^{b}	386^{c}	4.32	2.52^{a}	21.0
AFB1	218^{b}	0.35^{a}	126^{ab}	54.6	377	5.33^{a}	458^{a}	3.80	2.32^{b}	22.5
Savory	212^{bc}	0.29^{b}	126^{ab}	62.8	351	4.17^{b}	389^{c}	3.82	2.40^{b}	20.5
AFB1+ Savory	202^{c}	0.32^{ab}	123^{b}	61.6	356	4.33^{b}	410^{b}	4.01	2.38^{b}	21.8
SEM	2.9	0.082	2.0	2.08	7.1	0.173	16.5	0.096	0.021	0.54
Probability										
AFB1	**	**	NS	NS	NS	NS	NS	NS	**	NS
Savory	**	NS	NS	NS	NS	NS	NS	NS	NS	NS
AFB1× Savory	*	*	*	NS	NS	*	*	NS	*	NS

*^{a,b,c} Means within a column without a common superscript differ statistically (P<0.05); * P<0.05; ** P<0.01; **CHOL,** cholesterol; **TRG,** triglyceride; **AST,** aspartate aminotransferase; **ALT,** alanine aminotransferase; **ALP,** alkaline phosphatase; **TP,** total protein; **GGT,** gamma-glutamyltransferase*

Table 5. *Effect of savory on humoral immune response for broiler chicks fed diet containing 0.5 mg aflatoxin/kg at day 28 of age*

Tablo 5. *28. günde 0.5 mg aflatoksin/kg içeren rasyon ile beslenen broyler piliçlerde geyikotunun humoral immun yanıt üzerine etkisi*

Treatments	Newcastle (log2)	Influenza (log2)
Control	7.0[ab]	5.0
AFB1	5.0[b]	5.0
Savory	8.4[a]	5.6
AFB1+ Savory	6.2[b]	5.0
SEM	0.27	0.17
Probability		
AFB1	*	NS
Savory	*	NS
AFB1× Savory	*	NS

*[a,b] Values in the same row not sharing a common superscript differ significantly (P<0.05); * P<0.05; ** P<0.01*

body weight gain. In consistence with our results, Dorner et al.[16] reported that inclusion of aflatoxin in the diet of broiler chicks resulted in a decreased of daily weight gain. The reduced feed intake may be related to protein catabolism and an increase in the level of ammonia in blood. Moreover, the adverse effects of aflatoxin on growth performance have been related with a decrease in the protein and energy utilization [17], probably as a consequence of a deterioration of the digestive and metabolic efficiency of the birds.

The harmful effects of aflatoxin on the performance of birds were declined by addition of savory to aflatoxin contaminated diet. Also, in the total period birds fed savory alone had more weight gain and lower feed consumption and therefore better feed conversion ratio as compared to the control group. In consistence with our results, a study [18] showed that the addition of savory at the level of 200 mg/kg to diet of broilers could improve feed conversion ratio.

In this study the level of AST and ALP in serum, as markers of liver function, significantly increased in birds fed aflatoxin contaminated diet. Increase in the level of enzymes AST and ALP due to the addition of aflatoxin to diet has been reported by Dafalla et al.[19]. In contrast, there is a report [20] in which the addition of aflatoxin to diet has no effect on the level of these enzymes. These enzymes enter to blood from liver cells due to cell membrane damage [15]. By addition of savory to diet containing aflatoxin, the levels of AST and ALP decreased. In consistent with the results of the present study, there is a report [15], concerning the hepatoprotective effects of savory in rat that showed savory could decrease the levels of AST, ALT and ALP in serum. The major compounds in savory are the carvacrol and flavonoids [10,21]. Both of these compounds have antioxidant properties. These phenolic compounds present in the savory can prevent liver toxicity and thereby possibly reduce the release of liver enzymes into the blood. It is likely that the role of phenolic compounds present in savory is protection of the liver against toxic agents.

In the present study, the birds fed diet containing aflatoxin had lower concentrations of glucose and albumin as compared with the control group. Also, the total protein content of serum decreased as aflatoxin inclusion to the basal diet. These results agreed with the findings of previous studies [1,22], in relation to the reduction of serum total protein and albumin due to the presence of aflatoxin in diet of broilers. The reduction in total protein levels due to aflatoxin supply may result from a defection the transport of amino acids and mRNA transcription. Thus, the protein synthesis is prevented in the body of birds [23], which reflected in the serum as reduction in total protein.

In the birds fed diets containing aflatoxin the relative weights of the liver, spleen and pancreas increased as compared with the control group. The liver is a target organ for aflatoxins. It is possible that more detoxification process and more fat accumulation in the liver be main cause of increased the relative weight of liver. Significant increase in the relative weight of spleen in broilers fed diet contaminated with aflatoxin has been also reported by Bailey et al.[24]. An increase in the relative weight of the spleen can be attributed to an overactive of the spleen during feeding diets containing aflatoxins. Addition of savory to diet contaminated with aflatoxin decreased this effect on the relative weights of mentioned organs.

Serum antibody titer against NDV was the lowest in the birds fed diet containing aflatoxin. In consistent with our finding, in some of studies [25,26] chicks received aflatoxin treated ration had lower geometrical mean antibody titers against the Newcastle disease as compared to the control. Antibody titer against NDV significantly increased in group fed diet containing savory alone as compared with the control group. The savory has been reported to have antibacterial and antioxidant activities [6]. The major components savory, thyme and carvacrol have been indicated to possess potent antioxidant properties, and an increase in immune responses of chicks was anticipated. There was no report in the literature concerning the effect of savory on the immune response of animals.

The results of this study suggest that the addition of savory to diet could decrease the toxic severity of aflatoxins on broiler chicks. The protective action of this compound was particularly evident on growth performance. These findings provide a basis for further studies on the relationship between savory and protection against aflatoxins toxicity, to improve the safety and quality of poultry products.

Acknowledgments

The authors are grateful to the Islamic Azad University for research funding support. They thank Khoraman

Company for providing the savory extract and Animal Research Institute of Khoramabad for supplying the basal diet and allowing use of poultry unit. They also thank Ali Nikkhah for his assistance during laboratory works and all staffs in the poultry unit, for the assistance in the care and feeding of the chicks used in this research.

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