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THE PRODUCTIVE PERFORMANCE, INTESTINAL BACTERIA AND HISTOMORPHOLOGY OF BROILER CHICKS FED DIETS CONTAINING HOT RED PEPPER

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ABSTRACT: The current study aimed to investigate, the effect of feeding graded levels of hot red pepper on performance, ileal bacteria and histomorphology of broiler chicks. Four hundred day-old Ross broiler chicks were allocated into four groups. The chicks of the first group were fed basal diet (control) and the other three groups were fed the basal diet supplemented with three levels of red pepper as 0.5%, 1.0% and 2.0%. Body weight gain and feed conversion ratio improved significantly due to feeding different graded levels of hot pepper. Hot pepper has broad spectrum bactericidal activity against the growth of gram negative pathogenic bacteria E. coli, Enterobacteriaceae, and gram positive lactobacilli bacteria.

Plasma cholesterol and total lipids reduced significantly at level of 1% and 2% red pepper. Feeding 2% pepper increased plasma total protein and albumin significantly while, 0.5% and 1% levels lacked significance. Hot red pepper supplementation did not affect the dressing carcass, liver and heart weight percentage or immune organs spleen and bursa.

There were significant increments in intestinal villi length associate with significant reduction in crypt depth due to feeding hot pepper at 1% and 2% levels. It can be concluded that, adding hot red pepper into diets can improve broiler performance by increasing villi length and inhibiting harmful bacteria.

Key words: Broiler-hot pepper-performance-histomorphology-intestinal bacteria

INTRODUCTION

Capsicum genus (Capsicum annum L.) is a family of flowering plants includes hot red pepper and chilli pepper which usually used as appetizer in human diets (Al-Kassie *et al.,* 2011). Due to its bioactive components capsaicin, capsicum genus plants have been identified as medicinal plants (Puvaca, 2018; Abdelnour *et al.,* 2018). Capsaicin compounds are responsible for pungent and irritating effect of hot red pepper and other capsicum genus plants (Jancso *et al.,* 1997; Fattori *et al.,* 2016). Capsaicin is a group of compounds called capsaicinoids which include dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin (Fattori *et al.,* 2016). In addition, hot red pepper is rich in vitamin C and Pro vitamin A (β Carotene) both has antioxidant and antistressor properties (Lee *et al.,* 2005; Puvaca *et al.,* 2019; Puvaca, 2018). Recently, chemotherapeutics and chemopreventive properties of hot red pepper have been received great consideration in poultry nutrition domain (Puvaca *et al.,* 2015). Furthermore, hot red pepper has been known as photobiotic feed additives among broiler nutrition research (Al Kassie *et al.,* 2011; 2012). Previous studies indicated that, administration of hot red pepper into broiler diets improved feed intake, body weight gain and feed efficiency (El Deek *et al.,* 2012; Aghil shehaved *et al.,* 2013). The mode of beneficial action of capsicum plants in poultry nutrition may be related to capsaicin which has bactericidal effect against intestinal pathogens, E. coli, clostridium and salmonella (Agrawal *et al.,* 2017; Omola *et al.,* 2014; Tallez *et al.,* 1992). As well, capsaicin has the ability to protect gastrointestinal mucosal layer against injuries due to drugs or irritation agents (Tallez *et al.,* 1993; Al-Kassie *et al.,* 2012).

In spite of poultry do not sense the pungent effect of capsicum due to lake of receptors specific for capsaicin binding (Mason and Marunaik, 1983; Puvace *et al.,* 2015) that, allow using high ratios of capsicum in broiler diets. Most of previous investigators conducted their studies with adding little or tiny ratios of hot red pepper into their experimental diets which may not enough to pronounce the mode of action clearly. Aghil Shehaved *et al.* (2013) concluded that, the mode of beneficial action of hot red pepper is not understood yet. Therefore, the current study aimed to investigate, the effect of adding proportionally higher levels of hot red pepper into broiler chick diets on their performance. As well, its effect on intestinal bacteria and histomorphology were studied in an attempt for revealing the mode of action of hot pepper.

MATERIALS AND METHODS

Experimental Procedure: The current study was conducted at experimental poultry farm, animal production department, Faculty of Agriculture Science and Nutrition, King Faisal University, Saudi Arabia kingdom. For 42 days, four hundred day-old Ross broiler chicks were allocated randomly into four treated groups with fife replicates of 20 birds each. The chicks were fed basal diet contains 21% protein and 2850 Kcal ME. The first group was fed the basal diet which considered as control (T1). The other three groups were fed the basal diet supplied with three different graded levels of dried hot red pepper meal as 0.5%, 1% or 2%. The basal diet was formulated to meet the requirements of broiler chicks according to NRC (1994) and shown in Table 1. Hot red pepper was purchased as dried fruits from local market of El Hasa City, Saudi

Arabia Kingdom, and grinding before adding into diets. Water and mash feed were provided *ad lib*. The chicks were housed in floor pens with wood-shavings litter. Electrical heaters were used for warming, Fan and air conditions were used for keeping suitable temperature. Artificial lighting was provided constantly for 24 hrs. Body weights was recorded weekly for each chick and the average was calculated for each replicate and treatment group. Feed consumption was recorded weekly in gram, and feed conversion ratio was calculated as gram feed /gram gain.

Carcass Traits and Samples Collection: At the end of experimental period, 10 chicks per treated group were slaughtered, allowed to bleed for blood sample. Internal organs were separated. Dressed carcass, liver, heart spleen and bursa of fabricus weights were recorded in grams. Dressed carcass was calculated as percentage of live body weight, while heart and spleen was related to dressed carcass weight. Ileal content samples were collected in clean sterile glass bottle. Small intestine thickness was determined as the procedures described by Stutz *et al.* (1983) and calculated as: small intestine weight (g) / small intestine length (cm).

Blood analysis: Blood samples were collected and centrifuged at 4000 rpm for 15 minutes. Plasma total protein was determined according to Biuret method, (Henery ,1964) and albumin according to Doumas *et al.*, (1971). Serum globulin was calculated by subtracting albumin from total protein. Serum total lipid was determined according to Knight *et al.* (1972) and total cholesterol according to Watson (1960).

Intestinal bacteria: For microbiological examination, one gram of ileal content was transferred into test tube containing 9 ml of 0.1 sterile peptone. The samples were mixed well; tenfold dilution were prepared and grown on the following media:

Total aerobic bacteria were cultured on nutrient agar medium composed of (per liter) yeast extract 2.5g; trypton 5g, glucose 1g, agar 15g and distilled water up to one liter.

Lactobacilli bacteria were cultured on M.R.S. agar medium which is composed of casein peptone 10g, meat extract 10g, yeast extract 5g, glucose 20g, tween80 1g, K2mpo4 2g, sodium acetate 5g, diammonium citrate 2g., MnSO4 0.2g and distilled water up to 1 liter.

E. coli bacteria were cultured on MacChonkey agar medium that is composed of pancreatic digest of gelatin 17g pancreatic digest of casein 1.5g, peptic of animal tissue 1.5g, lactose 10g, bile salts 1.5g, sodium chloride 5g, neutral red 0.03g, crystal violet 0.001g, agar 3.5g, and distilled water up to 1 liter.

Enterobacteria were cultured on MacChonkey agar No.2 medium that is composed of (peptone 20g., lactose 10g., bile salt 5g., sodium chloride 5.0g., neutral red 0.075g and agar, 12g per liter). Salmonella bacteria were cultured on S.S. agar. Bacterial count was determined by microscopic examination of the cultured media.

Histomorpholgical examination: Small portion (2.5cm) of slaughtered birds ileum was dissected and placed in 10% buffered neutral formalin for fixation. A microtome was used to make 5µ sections that were mounted on glass slides and stained with hematoxylin and eosin. Villi length was measured from the apical to the basal region which corresponded to the superior portion of the crypts of Lieberkuhn by using light microscope

fitted with a digital camera and images were analyzed using image analysis software

Statistical analysis: Analysis of variance was carried out using statistical program SAS (1988). Duncan's multiple range tests (1955) was applied for significant differences among means of traits. The following model was used: $Y_{ij}= \mu + T_i + e_{ij}$. Where Y_{ij} = observation, μ = overall means, T_i = effect of treatment and e_{ij} = experimental error.

RESULTS AND DISCUSSION

The performance aspects of broiler chicks fed diets with different levels of hot red pepper are shown in table (2). At the first stage of growth (0-3 wk.), inclusion of red pepper by 2% significantly ($P < 0.05$) improved body weight gain compared to control. At the end of the second fattening period (0-6 wk), the improvement in body weight gain was clear and significant due to addition different levels of hot red pepper. The current results are in agreement with those of Puvaca *et al.* (2015) who did not observe any significant differences in body weight of chicks at the preparatory stage of growth by adding red pepper at 0.5 or 1% while, the increment was significant at final stage of growth. Inclusion of red pepper at level of 2% was more effective in increasing body weight gain without any deteriorate effect on chicks. William and Klenholz, (1974), stated that inclusion of hot pepper by level higher than 2%, was not toxic and had no significant effect on mortality rate. There were insignificant ($P < 0.05$) slight increments in cumulative feed intake (0 – 6 wk) due to adding different levels of hot pepper into broiler diets. Similar results were obtained by El-Deek *et al.* (2012) who did not observe any significant differences in feed intake of chicks fed hot red pepper. The slight increment in feed intake may be related to increase appetite, through the effect of capsaicin in stimulating energy metabolism as the result of activating the sympathetic nervous system (Kawada *et al.*, 1988). Feed efficiency results were parallel with those of body weight gain where inclusion level of 2% achieved the best ratio and differ significantly ($P < 0.05$) than control in both stages of growth. This finding is in harmony with those of El-Deek *et al.* (2012) and Al-Kassie *et al.* (2012) who observed an enhancement in feed conversion ratio due to inclusion hot pepper into broiler diets. Generally, the significant improvements of feed efficiency and body weight gain may be related to the bactericidal effect of capsaicin against intestinal pathogens which is reported herein (Table 3). In addition to improve whole tract nutrients digestibility via potentiate the activities of pancreatic and intestinal enzymes (Platel and Srinivasan 2004), increase bile acid secretion (Abdel Salam *et al.*, 2005) and maintain the intestinal mucosa (Al-Kassie *et al.*, 2012). Furthermore, the improvement may be related partly to the reduction in heat stress resulted from high content of vitamin C (Henken, 1991).

Effect of hot red pepper on some bacterial strains of ileum is shown in (Table 3). Inclusion of hot pepper into broiler diet reduced the growth of gram negative pathogenic bacteria E. coli and Enterobacteriaceae, the reduction was significant ($P < 0.05$) for E. coli. As well, hot pepper supplementation significantly inhibited the growth of gram positive lactobacilli bacteria. The current results proved that hot red pepper has broad spectrum bactericidal activity against both of the pathogenic or nonpathogenic bacteria either gram positive or negative. This finding is in agreement with the

results of Corduk *et al.* (2013) who stated that, capsaicinoids of hot pepper possess antimicrobial activities against pathogenic E. coli and Enterobacteriaceae strains. Abdul Aziz (2010) suggested that, the capsicum pepper has a broad spectrum effect on isolated bacterial strains due to bacteriostatic and bactericidal activity of capsaicin derivatives, t-cinnamic, and caffeic acids respectively.

Plasma total cholesterol depressed significantly ($P < 0.05$) due to inclusion hot red pepper into broiler diets at level of 1% and 2% (Table 4). As well, plasma triglycerides level reduced significantly at level of 2% red pepper, while the differences were insignificant at the levels of 0.5% and 1%. Several reports have been established the effect of capsaicin and hot peppers in reducing blood cholesterol levels in broilers (El-Deek *et al.,* 2012; and Adedoyin *et al.,* 2019). The hypocholesterolaemic effect of hot pepper can be related to its role in stimulating the hepatic cholesterol-7-hydroxylase enzyme needed for converting cholesterol to bile acids and subsequently depleting blood cholesterol level (Puvača *et al.,* 2015; Adedoyin *et al.,* 2019). The reduction in plasma triglycerides was reported previously and related to decrease lipids absorption (El-Deek *et al.,* 2012) or due to inhibition of the Acetyl CoA syntheses enzyme that is necessary for the biosynthesis of fatty acids (Puvača *et al.,* 2015). Blood proteins derivatives values (Table 4) were not affected significantly ($P < 0.05$) by adding hot red pepper at 0.5% or 1% while the level of 2% increased plasma total protein and albumin significantly. These results are in harmony with those obtained by Kist *et al.* (2011), and Corduk *et al.* (2013), who did not observe any significant effect on serum levels of total proteins, albumin and globulins by adding hot peppers into broiler diets. The significant increment in total protein and albumin resulted from 2% inclusion of hot red pepper may be related to uninvestigated intestinal irritation or hepatic disorder due to high inclusion of hot pepper. In this concern data is still lacking in poultry.

Hot red pepper supplementation did not affect the dressing carcass, liver and heart weight percentages (Table 5). As well, the weight of immune organs (Table 5) spleen and bursa did not differ significantly by feeding different levels of peppers except 0.5% level which increased bursa weight significantly. These results are in consist with those of Al-Kassie *et al.* (2011), Islam *et al.* (2018) and Daniel *et al.* (2017) who did not detect any significant differences in giblets weight due to feeding capsicum. As well El-Deek *et al.* (2012) stated that, spleen and bursa weight did not differ due to adding hot red pepper into broiler diets. Villi length significantly ($P \leq 0.05$) improved due to adding hot red pepper by 1% and 2% into diets (Table 6; Fig 1). Villi length increment was associated with significant reduction in crypt depth. Accordingly, the ratios of villi length to crypt depth were significantly higher by including different graded levels of red pepper into broiler diets. The present results are in harmony with those of Madhupriya *et al.* (2018) who stated that, phytogenic feed additive derived from capsaicin affect villi height and crypt depth in the jejunum of chicks. Aghil Shahverdi *et al.* (2013), and Cardoso *et al.* (2012), reported that adding red or black pepper into broiler diets can modify the morphology of small intestine by reducing the growth of pathogenic or

nonpathogenic intestinal organisms. These reductions in pathogenic bacteria reduce the inflammatory reactions at the intestinal mucosa leads to the increase of the villus area and improve functions of secretion, digestion and nutrients absorption. The improvement in nutrients utilization ultimately increases the efficiency of feed utilization and body weight gain which was reported herein (Table 2). Small intestine thickness was not affected due to feeding different graded levels of hot red pepper. This results disagreed with those of Aghil Shahverdi *et al.* (2013), who observed a significant increment in mucosa and sub mucosa thickness of small intestine by using hot red pepper in broiler diets. The disagreement may be related to different experimental conditions or level of red pepper inclusion.

CONCLUSION

Relying on the results of the present study, it may be concluded that, adding hot red pepper by 2.0% into broiler diets improve performance of broiler chicks. The mode of beneficial action of hot pepper may be related to its effect in improving the nutrients utilization via increase villi length and reduce the count of harmful bacterial strains**.**

Table (1) : Composition and calculated analysis of the Basal diet.

Ingredients	**%Basic Diet**
Yellow corn	60
Soybean meal (48%)	31.5
Wheat bran	5.5
Dicalcium phosphate	1
Limestone	1.3
Common salt	0.25
Vit. & min. premix*	0.33
DL. Methionine	0.12
'Total	100
Calculated analysis	
Crude protein%	21.24
M.E. Kcal/kg	2850
% Calcium	0.82
%Available phosphorus	0.315
%Methionine + cystein	0.68
%Lysine	1.12

*Composition of vitamin and mineral premix. Each 2.5 kg of vitamin and mineral mixture contains: 12000000 IU vitamin A; 2000000 IU D_3; 10g E ; 1g K ; 1 g B_1 ; 5g B_2; 1500mg B_6 ; 10mg B_{12} ;10g Pantothenic acid ; 20g Nicotinic acid ; 1g Folic acid ; 50mg Biotin ; 500 g choline chloride ;4 g copper ; 300 mg iodine ; 30g iron ; 60 g Manganese; 50g Zinc; and 100mg selenium

Table (2) : Effect of feeding graded levels of hot red pepper on performance of broilers.

Performance Aspects		**Control**	**Hot red pepper**		
			0.5%	**1%**	**2%**
Body weight gain (g/b)	(0-3)wk.	581.7^{b} ±16.4	610.5^{b}±6.5	598.8^{b}±15.12	665.3^{a}±12.85
	(0-6) wk.	1922.8^{c}±68.32	2136.4^{ab}±21.38	2106.3^{b}±30.02	2235.0^{a}±16.29
Feed intake (g/b)	(0-3) wk.	945.9^{b} ±31.3	980.1^{ab} ±21.2	988.1^{ab}±20.3	1024.2^{a}±15.1
	(0-6) wk.	3436.7±69.1	3701.3 ±125.2	3570.6 ±64.2	3712.98±60.5
Feed conversion ratio (g. feed/g. gain)	(0-3) wk.	1.63^{a}±0.03	$1.61^{a\ b}$±0.02	1.65^{a}±0.02	1.54^{b}±0.03
	(0-6) wk.	1.79^{a}±0.03	1.73^{ab} ±0.04	1.70^{b}±0.01	1.66^{b}±0.02

Means ± (Standard error) Values within a raw with different superscripts are significantly different (P≤0.05)

Table (3): Effect of feeding broilers on graded levels of hot red pepper on ileal bacteria.

Bacterial Strains	**Control**	**Hot red pepper**		
		0.5%	**1%**	**2%**
Lactobacilli sp. (CFU/g)	$9.7\times10^{6\,a}\pm0.38$	$1.68\times10^{6\,b}\pm0.04$	$2.2\times10^{6\,b}\pm0.11$	$5.3\times10^{6\,b}\pm0.19$
E. coli (CFU/g)	$15.3\times10^{5\,a}\pm0.58$	$4.4\times10^{5\,b}\pm0.16$	$6.2\times10^{5\,b}\pm0.28$	$3.6\times10^{5\,b}\pm0.13$
Enterobacteriaceae (CFU/g)	$6.2\times10^{6}\pm0.52$	$5.4\times10^{6}\pm0.25$	$3.9\times10^{6}\pm0.17$	$3.6\times10^{6}\pm0.19$
Total bacteria (CFU/g)	$7.9\times10^{7}\pm0.54$	$5.8\times10^{7}\pm0.78$	$6.1\times10^{7}\pm0.89$	$2.1\times10^{7}\pm0.15$

Means ± (Standard error) Values within a raw with different superscripts are significantly different ($P\leq0.05$)

Table (4) : Effect of feeding broilers on graded levels of hot red pepper on some blood constituents

Blood Constituents	**Control**	**Hot red pepper**		
		0.5%	**1%**	**2%**
Cholesterol (mg/dl)	$152.58^{a}\pm 4.165$	$150.56^{a}\pm 2.708$	$142.02^{b}\pm 1.167$	$140.65^{b}\pm 2.107$
Triglyceride (mg/dl)	$30.3^{a}\pm 0.84$	$28.7^{ab}\pm 0.52$	$28.7^{ab}\pm 0.82$	$26.4^{b}\pm 0.79$
Total Protein (mg/dl)	$3.50^{b}\pm 0.076$	$3.95^{ab}\pm 0.110$	$3.44^{b}\pm 0.066$	$4.43^{a}\pm0.095$
Albumin (mg/dl)	$2.13^{cb}\pm 0.103$	$2.50^{b}\pm 0.082$	$2.07^{c}\pm 0.047$	$2.95^{a}\pm 0.120$
Globulin (mg/dl)	1.367 ± 0.098	1.447 ± 0.05	1.367 ± 0.044	1.149 ± 0.080

Means ± (Standard error)
Values within a raw with different superscripts are significantly different ($P\leq0.05$)

Table (5) : Effect of feeding broilers on graded levels of hot red pepper on carcass traits of broilers at 42 days of age

Carcass traits	**Control**	**Hot red pepper**		
		0.5%	**1%**	**2%**
Dressing %	70.9 ± 1.5	69.3 ± 1.1	73.3 ± 1.8	72.5 ± 2.1
Liver to Wt. %	3.24 ± 0.19	3.19 ± 0.17	2.90 ± 0.11	2.91 ± 0.13
Heart wt. %	0.73 ± 0.02	0.69 ± 0.05	0.63 ± 0.03	0.66 ± 0.04
Spleen wt. (g)	3.11 ± 0.172	2.69 ± 0.295	2.92 ± 0.239	3.11 ± 0.182
Bursa wt. (g)	$1.86^{b}\pm0.120$	$2.45^{a}\pm0.213$	$1.8^{b}\pm0.168$	$1.93^{ab}\pm0.164$

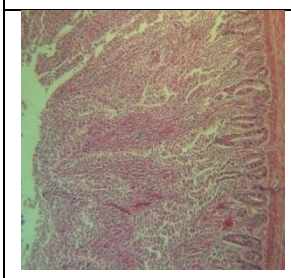
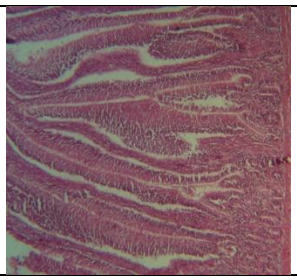
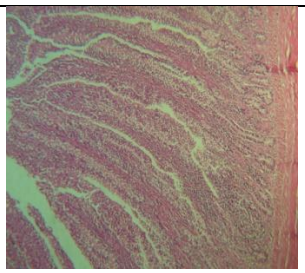
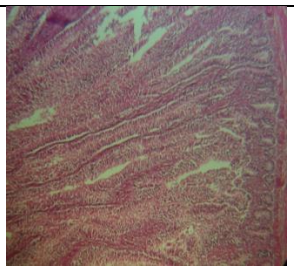
Means ± (Standard error)
Values within a raw with different superscripts are significantly different ($P\leq0.05$)

Table (6) : Effect of feeding broilers on graded levels of hot red pepper on ileal histomorphology of broilers

Traits	Control	Hot red pepper		
		0.5%	1%	2%
Villi length	467.67^{c}±17.41	514.8^{bc}±18.44	534.88^{b}±7.60	599.50^{a}±11.61
Crypt depth	127.25^{a}±4.58	98.20^{b}±5.26	106.88^{b}±6.35	107.70^{b}±6.21
Villi / Crypt ratio	3.75^{b}±0.227	5.45^{a}±0.351	5.13^{a}±0.312	5.72^{a}±0.323
Small intestine thickness (g wt/cm length)	0.209±0.009	0.205±0.008	0.213±0.005	0.202±0.007

Means ± (Standard error)
Values within a raw with different superscripts are significantly different (P≤0.05)

Fig (1): Ileal histomorphology show villi length of control and hot red pepper feeding groups

Control	Hot Red Pepper		
	0.5%	1%	2%
			

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الملخص العربى

الأداء الأنتاجى وبكتريا وهستومورفولوجى الأمعاء فى كتاكيت اللحم المغذاة على الفلفل الأحمر الحار

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تهدف الدراسة لمعرفة تأثير تغذية كتاكيت اللحم على مستويات متدرجة من الفلفل الأحمر الحار على الأداء الأنتاجى هستومورفولوجيا وبكتريا الأمعاء. الدراسة أستخدمت عدد 400 كتكوت روس عمر يوم تم توزيعهم على أربع معاملات. المعاملة الأولى غذيت على عليقة قاعدية (كونترول) والثلاث معاملات الأخرى جرى تغذيتها على العليقة القاعدية مضاف اليها ثلاث مستويات متدرجة من الفلفل الحار هى 0.5% و 1.0% و2.0%. أشارت النتائج الى تحسن وزن الجسم المكتسب ومعدل التحويل الغذائى للكتاكيت المغذاة على النسب المختلفة من الفلفل الأحمر. الفلفل الأحمر له تأثير واسع المدى ضد البكتريا الممرضة السالبة لجرام من نوع بكتريا القولون والبكتريا المعوية كذلك ضد البكتريا الموجبة لجرام من نوع اللاكتوباسلس. محتوى بلازما الدم من الكوليسترول والليبيدات الكلية أنخفض بشكل معنوى للتغذية على مستويات 1.0% و 2.0% فلفل. أضافة الفلفل بمستوى 2% أدى لرفع معدل البروتين الكلى والألبيومين فى الدم دون تأثير لباقى المستويات. لم يكن هناك تأثير لأضافة الفلفل الأحمر على النسب المئوية لصافى الذبيحة أو العضاء الداخلية (كبد – قلب) أو على وزن الأعضاء المناعية الطحال والبرزا. هناك زيادة معنوية فى طول خملات الأمعاء مصحوبا بنقص فى تجويف كريبت نتيجة للتغذية على الفلفل الأحمر بنسبة 1.0% و 2.0%.

وقد خلصت الدراسة الى أن أضافة الفلفل الأحمر الحار لعلائق كتاكيت اللحم عند مستوى 2% يحسن من الأداء الأنتاجى نتيجة لتأثيره النافع فى تحسين بيئة الهضم والأمتصاص من خلال خاصيتى طول الخملات ومسطح الأمتصاص وتأثيره المضاد للبكتريا الضارة فى أمعاء الطيور.

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