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Response of broiler chicks fed by clove and cardamom alcoholic extracts

Resposta de pintos de corte alimentados com extratos alcoólicos de cravo e cardamomo

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Abstract: This study investigated the effectiveness of clove and cardamom alcoholic extracts as growth promoters on performance, carcass traits, some blood serum components, immunity parameters, intestinal microbial population and intestinal morphology of broiler chicks. To this purpose, a total of 240 one-day-old broiler chicks (Ross 308 strain) were distributed randomly into three groups in a form of completely randomized design. The experimental groups were subdivided into four replicates with 20 chicks each. The experiment was continued 6 weeks. Chicks were fed by basal diet as control, basal diet contain $300_{(ml.ton)}$ clove and basal diet contain $300_{(ml.ton)}$ cardamom alcoholic extract. The result showed that significant increase in FI and BW in clove and cardamom groups while FCR decreased significantly. Using these herbal extracts already reduced abdominal fat and bursa of fabricius percentage while liver and spleen weight percentage increased significantly. Triglyceride and cholesterol levels decreased in treatments and high-density lipoprotein HDL increased. Also, low density lipoprotein LDL level decreased significantly in clove and cardamom groups. As result reveled antibody titers (ND and SRBC) increased significantly in treated groups compared to the control. It was showed that clove and cardamom extracts had significant effects on intestinal microbial population compared to control group. Data showed that Escherichia coli population decreased while Lactobacillus increased significantly compared to the control. We could demonstrate that inclusion of clove and cardamom extracts may useful and have beneficial effects on performance, blood serum components, values of immunity parameters and intestinal morphology in experimental Ross 308 broiler chicks.

Key words: Broiler, Blood serum components, Cardamom, Clove, Intestine, Performance.

Resumo: This study investigated the effectiveness of clove and cardamom alcoholic extracts as growth promoters on performance, carcass traits, some blood serum components, immunity parameters, intestinal microbial population and intestinal morphology of broiler chicks. To this purpose, a total of 240 one-day-old broiler chicks (Ross 308 strain) were distributed randomly into three groups in a form of completely randomized design. The experimental groups were subdivided into four replicates with 20 chicks each. The experiment was continued 6 weeks. Chicks were fed by basal diet as control, basal diet contain 300(ml.ton) clove and basal diet contain 300(ml.ton) cardamom alcoholic extract. The result showed that significant increase in FI and BW in clove and cardamom groups while FCR decreased significantly. Using these herbal extracts already reduced abdominal fat and bursa of fabricius percentage while liver and spleen weight percentage increased significantly. Triglyceride and cholesterol levels decreased in treatments and high-density lipoprotein HDL increased. Also, low density lipoprotein LDL level decreased significantly in clove and cardamom groups. As result reveled antibody titers (ND and SRBC)

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1. Introduction

Herbal, as feed additives are possible natural alternatives to the use of antibiotics and probiotics as growth promoters in broiler diets plant active principles are chemical compounds present in the entire plant or in specific parts of them that confers them therapeutic activity or beneficial effects (Martins.2000).

In these context, Gianneanas et al .2003 state these substances are derived from the plant secondary metabolism, including glucosides, alkaloids phenolic and polyphenolic compounds, terpenoids, saponins, mucilages, flavonoids, and essential oils . A number of studies have reported that the beneficial effect of these spices or their active components on digestion process in broilers (Great head.2003). Herbal plant extracts have received increased attention as possible antibiotic growth promoter replacements. On a closer look, aromatic plants and their essential oils extracted became interesting due to their antimicrobial, antioxidant effects and stimulating effects on animal and poultry performance and digestive enzymes (KHEIRI et al .2014).

Clove (*Eugenia caryophyllata*) considers as a good source of manganese, calcium, magnesium dietary fiber, C and K vitamins and omega 3 fatty acids (MEHR et al .2014). Besides, clove extract contains different molecules (mainly eugenol) that have intrinsic bio-activities on animal physiology and metabolism. Clove has been used in the food industry because of its special aroma and natural safety in comparison to artificial feed additives (AGOSTINi et al .2012). Also, the essential oil of clove showed very strong antibacterial properties and performance enhancer.

Hernandez et al (2004) shines a line on the preventive effect of clove on the stress induced biochemical changes indicating its anti-stress activity.

Cardamom (*Elettaria cardamomum*) has been reported to possess antioxidant, anti-microbial, anti-inflammatory, for indigestion, appetite stimulant, carminative and it has good effects in poultry nutrition (SINGH et al .2008). Dietary cardamom improved the overall performance of broiler chicks and this may be attributed to the digestion influence on wall mucus and gastric acid (JAMAL et al .2005).

A number of researches showed cardamom extracts stimulate superoxide dismutase is an antioxidant enzyme leading to decreased lipid per oxidation (RUIZ et al .1999). As Sang et al (2013) pinpoints, the cinnamon powder may improve the performance and chicken meat with maximize the productivity of broiler chickens. Omidi et al (2014) demonstrated that using of cardamom essential oils had a positive effect on the blood cholesterol and triglyceride profile by decreasing the plasma cholesterol and LDL levels in broiler chicks. Osman et al (2005) believe that clove extract improved the digestibility of the feeds for broilers.

Accordingly, Mukhtar (2011) showed that chicks fed on 600mg.kg clove oil recorded more in feed intake and body weight compared to the control groups. Also (Osman et al .2005) noted that different essential oils were added to poultry diets could improve feed intake and feed conversion ratio and carcass yield. For as much as clove and cardamom extract have a wide range and potential uses, the aim of this study was to determine the efficacy of clove and cardamom alcoholic extracts on performance, carcass traits, some blood serum components, immunity parameters and intestinal morphology of broiler chicks.

2. Material and methods

This experiment was conducted at the poultry farm of Vardanjan town, Shahrekord city, Iran. (Coordinates: 32°19′N, 50°48′E and above mean sea level elevation: 2061 m). All experimental procedures were in according with established standards for the care and use of animal's rights for research purposes.

2.1. Experimental birds and design

240 one-day old commercial broiler chicks (Ross 308) were weighed and distributed randomly into 12 floor pens in a completely randomized design (20 birds per each pen). The average weight of each chick

was 39±55g and the pens were then divided randomly among four experimental diets. Feed samples were analyzed according to (AOAC .2000) for their chemical components and balanced based on the (NRC .2001). Chicks were fed by basal diet as control, basal diet contained $300_{(ml.ton)}$ clove and basal diet contained $300_{(ml.ton)}$ cardamom alcoholic extract (Table-1) for about 6 weeks. All birds were reared in deep litter with feed and water supplied as an adlibitum.

Table 1 – Composition of the experimental diet

Ingredients %	**0-02 (weeks)**	**02-04 (weeks)**	**04-06 (weeks)**
Maize grain	51.64	56.61	60.37
Soybean meal	37.74	32.30	27.81
Wheat grain	5	5	5
Soybean Oil	1.40	2.03	2.84
DCP	1.56	1.47	1.39
Oyster shells	1.17	1.13	1.08
Methionine D-L	0.30	0.29	0.27
Lysine-L	0.13	0.13	0.30
Edible Nacl	0.26	0.24	0.14
Vitamin Premix*	0.3	0.3	0.3
Mineral Premix*	0.3	0.3	0.3
Filler	0.2	0.2	0.2
Calculated nutrient content			
ME(Kcal.kg)	2.850	2.950	3.050
CP (%)	22	20	18.5
Ca (%)	0.90	0.85	0.80
Available Phosphorus (%)	0.45	0.42	0.40
Lysine (%)	1.35	1.20	1.16
Na (%)	0.16	0.15	0.15
Methionine+Cystine (%)	0.97	0.87	0.85

*Supplied Per Kilogram Of Feed: 7.500 IU of vitamin A, 2000IU vitamin D3, 30 Mg vitamin E,1.5 µg vitamin B12,2Mg B6,5 Mg Vitamin K,5 Mg vitamin B2,1 Mg vitamin B1,40 Mg nicotinic acide,160µg vitamin Biothine,12 Mg Calcium pantothenate,1MgFolic acid 20 Mg Fe,71 Mg Mn,100µg Se,37Mg Zn,6 Mg Cu,1.14 Mg I,400 µg Cu.

2.2. Management and Data collection

Feed intake, weight gain and feed conversion ration were measured weekly. At

the end of the experimental period 4 birds from each replicate of each dietary treatment were randomly selected, weighed individually then slaughtered and allowed to bleed. Samples of blood were collected into clean dry test tubes and allowed to clot and serum was separated, collected, frozen and later analyzed. Hot carcasses weight was recorded and the dressing percentage was determined by expressing hot carcass weight to the live weight.

2.3. Detection of antibody titer to ND and SRBC

The birds were vaccinated against Newcastle disease at 10 days (IB) and in 30 days (Lasota). Gambaro disease vaccine was given at the 22 days. Using of hemagglutination inhibition test (HI) according to (Beard .1989) with chicken red blood cells and four units of NDV (Newcastle disease vaccine) antigens, then geometric mean titers were calculated. For SRBC (Sheep Red Blood Cells) Immune assay, also four of the broilers within each pen were inoculated i.v. with 0.1 mL of 0.025% SRBC. The remaining broilers in a pen were inoculated with 0.1 mL of 0.25% SRBC, which was the dosage used in selection. Fresh sheep blood was collected from the Islamic Azad University of Shahrekord Branch Veterinary Hospital in sterile Alsevar's solution. At 3, 5, 7, 9, 11, 13, and 20 days after inoculation, 10 chicks from each line-dosage subclass were randomly chosen and bled (0.5 mL of blood). Blood was refrigerated for 24 h and plasma tested for total SRBC antibody by the procedure of (WEGMANN AND SMITHIES.1966). Antibody titers were expressed as the $\log^2$ of the reciprocal of the last dilution in which agglutination was observed macroscopically.

2.4. Blood serum components evaluation

Blood samples from each bird were collected and stored at refrigerator at $+4^{\circ C}$ for 24 ^{h} and then they were subjected to biochemical determine for their Serum lipids such as cholesterol, triglycerides, HDL and LDL toward method described by (Ellefson and Graway.1967) and (FRINGS and DUNN. 1970).

2.5. Intestinal microbial count

The internal organs were removed after slaughter. About 7 cm from the length of the ileum was sampled to determine the microbial population. Also 1 g of ileum content was used to make 10-fold dilution using buffered peptone water and then 0.1 mL of the appropriate ileum dilution was spread on Lactobacillus MRS1 Agar-Hi Media Laboratories to detect lactic acid bacteria and VRBA2 (Violet Red Bile Agar) to detect E. coli form. The cultures of

Lactobacillus and E. coli bacteria were made an aerobically form. The plates were incubated at 37.5$^{\circ C}$ for 48 h. After counting the number of colonies in each plate, the number so obtained was multiplied by inverse of the dilution and the result was stated as the number of colony forming unit (cfu) in 1 g of the sample as described by (DOWNES and Ito.2001).

2.6. Intestinal morphology

The small intestine histomorphometric examination was performed by light microscopy, and the measurement was done using public domain image analysis software (Image J, National Institute of Mental Health, Bethesda, MD, USA).

2.7. Statistical analysis

The data were analyzed based on the following model: $Y_{ij} = \mu + t_i + e_{ij}$

Whereas: Y_{ij} = Average effect observed, μ = Total average, t_i = Effect of treatments, e_{ij} = Effect of errors,

The GLM procedure of (SAS .2001) software was performed for data analysis of variance as completely randomized design. The significant difference among the mean were compared by (Duncan's multiple range tests.1995).

3. Result

According to Table 2 significant increase in FI and BW was observed in clove and cardamom groups while FCR decreased significantly ($p \leq 0.05$). Data from this study revealed that inclusion with clove and cardamom extract increase pre-slaughter weigh and dressing percentage significantly ($p \leq 0.05$).

Table 2 – The effect of added experimental diets on broilers performance

Treatments	BW(g.d)	FI(g.d)	FCR	FI (g)	Pre-slaughter weigh (g)	Carcass Yield (%)
Control	47.40^{b}	78.34^{b}	1.65^{a}	2233.75^{c}	1994.2^{c}	68.21^{c}
Clove	51.19^{a}	82.54^{a}	1.61^{b}	2341.25^{a}	2124.3^{a}	73.28^{a}
Cardamom	50.58^{a}	80.12^{b}	1.58^{b}	2248.20^{b}	2117.4^{b}	72.20^{b}
P value	**	**	**	**	**	**

*Means within row with no common on letter are significantly different ($p \leq 0.05$)

The effects of clove and cardamom alcoholic extract on some carcass traits of broiler chicks are showed in table 3. The results revealed that using herbal extract already reduced abdominal fat and bursa of fabricius percentage.

These treatments also significantly increased liver and spleen percentage (p≤0.05). There were significant differences about drumstick and breast meat percentage between treatments (p≤0.05).

Table 3 – The effect of added experimental diets on percentage of some visceral organs.

Treatments	Spleen (%)	Abdominal Fat (%)	Drumstick (%)	Breast (%)	Heart (%)	Borsal.f (%)
Control	2.49	4.55[a]	24.48[a]	25.30[a]	2.10	2.84[b]
Clove	2.74	3.21[b]	23.22[b]	26.36[b]	2.25	3.45[a]
Cardamom	2.75	3.50[b]	23.16[b]	25.90[b]	2.14	3.24[a]
P value	n.s	**	**	**	n.s	**

*Means within row with no common on letter are significantly different (p≤ 0.05)

The serum lipid profile of control and treated groups is given in Table 4. Triglyceride and cholesterol concentration decreased (p≤0.05). In herbal treatments while HDL increased. LDL concentration decreased significantly in clove and cardamom alcoholic extract groups (p≤0.05).

Table 4 – The effect of added experimental diets on blood serum components

Treatments	Triglyceride (Mg.dl -1)	Cholesterol ($Mg.dl^{-1}$)	HDL ($Mg.dl^{-1}$)	LDL ($Mg.dl^{-1}$)
Control	140.36[a]	130.20[a]	37.00[c]	139.19[a]
Clove	133.20[c]	125.00[c]	39.00[a]	133.00[c]
Cardamom	137.63[b]	126.75[b]	38.16[b]	136.00[b]
P value	**	**	**	**

*Means within row with no common on letter are significantly different (p≤ 0.05)

As Table 5 displays, Antibody titers (ND and SRBC) increased significantly (p≤0.05) in clove and cardamom compared to the control. It was showed that clove and cardamom extracts had significant (p≤0.05) effects on intestinal microbial population

compared to control group (Table 5). Data showed that E. coli population decreased while Lactobacillus population increased significantly in treated groups compared with the control group ($p \leq 0.05$).

Table 5 – The effect of experimental diets on antibody titer and intestinal microbial populations

Treatments	HI (log^2)	SRBC (log^2)	E. Coli ($cfu.g^{-1}$)	Lactobacillus ($cfu.g^{-1}$)
Control	2.00^c	5.33^c	7.22^a	4.78^c
Clove	2.75^a	6.75^a	6.30^c	5.69^a
Cardamom	2.25^b	6.50^b	6.48^b	5.32^b
P value	**	**	**	**

*Means within row with no common on letter are significantly different ($p \leq 0.05$)

According to the table 6, muscularis, serosa, goblet cell numbers and total length of intestine increased significantly in herbal treated groups compared to control group ($p \leq 0.05$). Also the broiler chicks fed with clove extract had thicker mucosa, muscle layer, serosa, and total than other groups.

Table 6 – The effect of experimental diets on intestinal morphology

Treatments	Mucosa (Micron)	Muscle layer (Micron)	Serosa (Micron)	Goblet cell (Number)	Total (Micron)
Control	110.15^c	12.00	6.95	5.50^c	128.05^c
Clove	114.24^b	12.25	7.01	6.25^b	130.50^a
Cardamom	112.24^a	12.14	7.00	6.00^a	129.10^b
P value	**	n.s	n.s	**	**

*Means within row with no common on letter are significantly different ($p \leq 0.05$)

4. Discussion

Previous studies demonstrated that clove and cardamom extracts or powders increase digestibility and performance in broiler chicks (AGOSTINI et al. 2011; JAMAL et al.2005). Also, various studies indicated an increase in body weight and feed conversion ratio by using clove or cardamom in broiler diet (MEHR et al. 2014; MUKHTAR .2011).

In the present study, feed intake, weight gain and feed conversion ratio improved in the treated groups ($p \leq 0.05$). The findings of the present study are consistent with some studies conducted in this field. Azadeganmehr et al (2014) demonstrated clove essence at the level of 450 ppm in finisher period increased feed intake, body weight gain and improved feed conversion ratio ($p \leq 0.05$).

The results of (Cabuk et al .2003) showed that ugeonol is considered as digestion stimulatory factors and the improvement of body weight gain and feed conversion are due to active materials found in clove essential oil causing greater efficiency in utilization of feed, resulting in enhanced growth. Results of (Raja et al.2001) study on broiler chicks showed that the dietary cardamom supplementation had significantly increased ($p \leq 0.05$) feed intake and body weight gain. A significant increase in body weight gain of broiler chicks fed clove and cardamom extract supplemented diet may be due to stimulant, essential oil content and anti-microbial activities of cardamom, this in agreement with findings of (KRITTIKA et al.2007 and FAGHANI et al.2014).

Although some researchers (Demir et al. 2003) and (Hernandez et al .2004) did not find statistical differences in the growth performance of birds which fed diets supplemented with different types or combinations of herbal extracts, but the results of current study were in line with those of (Osman et al. 2005 and Ghaedi et al. 2014) in which different essential oils and extract were added to poultry diets which improved FI, FCR and carcass yield.

Azadeganmehr et al (2014) showed that different levels of clove essential oil and probiotic had not any significant effect on dressing percentage, abdominal fat and internal organs percentage ($p \leq 0.05$). Ghaedi et al (2014) showed that liver and gizzard weight increased significantly ($p \leq 0.05$) in chicks that fed by black pepper extract. They also showed that drum stick and carcass yield percentage increased significantly in experimental treatments compared to control.

Lee et al (2004) showed that thymol and cinnamaldehyde at the level of 100 ppm stimulate secretion of pancreatic enzyme such as amylase, lipase, trypsin and chymotrypsin in broiler chickens and induce to increase their performance and better visceral organs percentage.

The result of this showed that clove and cardamom alcoholic extracts had significant ($p \leq 0.05$) effects on some immune parameters and intestinal microbial population. In the current study, the population of E. coli decreased significantly while the number of lactobacillus increased.

Clove and cardamom also are natural antioxidants, antibacterial, antiviral, antiparasitic and antifungal agent (Valero and Salmeron .2003; Singh et al .2002 and PINA VAZ et al. 2004). Kim et al (2004) showed that the higher number of antioxidants activities may help in improved immune status in broilers seriously.

As plant extracts could control and limit the growth and colonization of numerous pathogenic and nonpathogenic species of bacteria in the intestine, likewise, recent studies on supplementation of plant extracts to the broiler diets have supported our study with the similar results (CIFTC et al.2005; KHEIRI et al .2014 and GHAEDI et al .2014). Serum lipids decreased by using of clove and cardamom extracts may be due to antioxidant activity of them that decreases lipids per oxidation ($p \leq 0.05$).

According to (Azadeganmehr et al., 2013) results, dietary supplementation with 450_{ppm} clove essential oil could decrease blood serum cholesterol, LDL and improve HDL.LDL ratio ($p \leq 0.05$). Sang et al (2013) showed that cardamom essential oils supplementation had a beneficial effect ($p \leq 0.05$) on the blood cholesterol profile by decreasing the plasma cholesterol and LDL levels. Faghani et al (2014) found that using of turmeric extract in the diet of broiler chicks had good effects ($p \leq 0.05$) regarding the blood cholesterol profile and balancing the intestinal microbial population in the positive direction. Raja et al [26] showed that the cardamom feeding had significantly ($p \leq 0.05$) decreased blood total lipids and glucose in experimental chicks. The hypocholesterolemic action of herbals is possibly related to theirs phenolic and polyphenolic components.

They have been shown to depress the reverse-cholesterol transport and reduce the intestinal cholesterol absorption by increasing bile acid excretion (TEBIB et al., 1994). Some researcher noted that the main components of clove and cardamom essential oils inhibit hepatic 3- hydroxy-3 methylglutaryl coenzyme A reductase activity, which is a key regulatory enzyme in cholesterol synthesis (MUKHTAR .2011; OMIDI, et al. 2015). In addition, the reduction in blood cholesterol may be attributed to the reduction in some hormones secreted by the cortex of adrenal glands, which in turn causes the reduction in the secretion of fatty acids from adipose tissues or the reduction of fat oxidation.

The intestinal system was affected by clove and cardamom extracts ($p \leq 0.05$). According to (Samanya and Yamauchi.

2002) increased villus height is associated with increase digestive and absorptive function of the intestine due to the increased absorptive surface area and the expression of brush border enzymes. In fact, active ingredients in herbal are able to change and modify the morphology of the small intestine. This may be attributed that they reduced the growth of pathogenic or nonpathogenic intestinal organisms and by the fact the reduction of the inflammatory reactions at the intestinal mucosa leads to the increase villus area (MILES et al. 2006).

Kheiri et al (2014) reports demonstrated that herbal extracts can control microbial population by acting on the microflora's biochemical processes or inhibiting the elongation of gram-negative bacteria such as Escherichia coli, or by reducing lactic acid producing bacteria in the gastrointestinal tract. Lactobacillus, which has a high bile salt hydrolytic activity and it, is responsible for deconjugation of bile salts (Sarono.1995). Deconjugated bile acid is less soluble at low pH and less absorbs in the broiler intestinal system (KLAVEr and VAN DER MEER. 1993)

5. Conclusions

It is concluded that dietary supplementation of clove and cardamom alcoholic extract could reduce triglyceride, cholesterol, and LDL in plasma of broiler chicks at 42 days. The results of present study drew attention to the fact that the use of clove and cardamom alcoholic extract may reduce the intestinal pH and provide a good environment for Lactobacillus growth and limited multiplication of Escherichia coli bacteria. Furthermore, it can be suggested that increased integrity of the intestinal tract associated with a greater mucosa, muscle layer, serosa, as well as increase goblet cells number and total length of intestine after clove and cardamom alcoholic extract supplementation can result in improved performance, carcass traits of broiler chicks.

Also, we have found that antibody titer against ND and SRBC were improved by these herbals. These improvements are associated with their biological functions, role as stimulant of feed digestibility, anti-oxidant, anti-microbial or prevention of gastric toxicity. The mechanisms by which these herbals influence the performance, immune system and gut microflora of broilers are not known properly, therefore further studies are needed for more explanation.

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