



بسم الله الرحمن الرحيم

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Effect of added levels of Clove (*Eugenia aromaticum*) extract on the performance, some immune response, Blood Constituents and some Carcass characteristic of broiler chicks

أثر إضافة مستويات من مستخلص القرنفل على الأداء وبعض الصفات المناعية ومكونات الدم وبعض خصائص الذبيح في الدجاج اللحم

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

وَلَحْمِ طَيْرٍ مِّمَّا يَشْتَهُونَ ﴿21﴾

صدق الله العظيم
سورة الواقعة الآية (21)

Dedication

To my Father's Soul

To my Mather's

To my Sisters

To my Colleagues and Friends

With best Wishes

Acknowledgement

First of all my thanks and praise to almighty Allah, the beneficent, the merciful for giving me health and strength to accomplish this work. Without help of some people this research would n't come out. My special thanks to Professor: Intisar Yousif Turki for his support, advice and guidance; this study could not have been completed without him. My thanks are also extended to anyone who helped in accomplishing this work.

Abstract

This study was conducted to evaluate the response of broiler chickens to adding graded levels (0g/l -250g/l -500g/l -750g/l) of clove extract in drinking water. The study measured included production performance, immune response, some carcass characteristics, attributes and economic evaluation impact.

The experiment birds were randomly distributed into four treatments using a completely randomized design. 200 one day old chicks (Cobb500) were used. They were divided into four treatments, each containing 50 chicks, and each treatment consists of five replicate 10birds each.

The experimental feed was formulated to be isocaloric and isonitrogenous to meet the nutritional requirements of broiler chickens issued from NRC (1994), the experimental chicks were fed for 5 weeks as experimental period and at the end two birds for each replicate were taken for slaughtered measurements. Results of this study showed that added clove to drinking water chicks was revealed a significant improvement in the percentage of feed consumed, total weight gain and carcass weight. The results showed that a highly significant differences ($P<0.01$) among experimental groups in feed intake, weight gain and final body weight gain. Group B of 250g/l clove level concentration was superior values for above parameters while significant improvement of ($P<0.05$) were recorded by group drinking clove extraction. Also addition of clove extraction appeared a significant improvement of economical impact. Percentage of concentration of 250g/l was given a highest values among the experimental groups while 750g/l is most profitable. Therefore, cloves can be introduced as a natural feed additive in the drink water of broiler chickens.

ملخص الدراسة

أجريت هذه الدراسة لتقييم استجابة الدجاج اللحم لإضافة مستويات متدرجة (0 g/l-250g/l-500g/l-750g/l) من مستخلص القرنفل في مياه الشرب. وشملت الدراسات قياسات الاداء الإنتاجي, والاستجابة المناعية, وبعض خصائص الذبيح والتقييم الاقتصادي. تم تصميم التجربة باستخدام التصميم العشوائي الكامل. تم استخدام 200 كتكوت عمر يوم واحد من سلالة كوب. قسمت الى أربع مجموعات, كل مجموعة تحتوي على 50 كتكوت, موزعة على اربع معاملات, كل معاملة تحتوي على خمس مكررات. تم تركيب العليقة التجريبية لتكون متساوية البروتين ومتساوية في الطاقة لتقابل الإحتياجات الغذائية للدجاج اللحم وفقاً ل(NRC (1994), تم تغذية المجموعة التجريبية لمدة 5 أسابيع عند نهاية التجربة تم أخذ طائران عشوائياً لكل مكرر ووزنا ثم ذبحا. أظهرت نتائج هذه الدراسة ان إضافة القرنفل الى كتاكيت مياه الشرب أظهرت إختلافاً معنوياً في نسبة العلف المستهلك, الوزن المكتسب الكلي للجسم ووزن الذبيحة. أظهرت النتائج وجود فروق ذات دلالة إحصائية عالية ($P < 0.01$) بين المجموعات التجريبية في تناول العلف, وزيادة الوزن, وزيادة وزن الجسم النهائي. وأظهرت المجموعة B (250g/l) تفوق عن بقية المجموعات للمعاملات المذكورة اعلاه. كما أظهرت النتائج أن إضافة مستخلص القرنفل أدى الى تحسناً كبيراً في التقييم الاقتصادي. بينما إضافة نسبة (250g/l) في المجموعة هي الأعلى ربحية بين مجموعات الإختبار. لذلك يمكن إدخال القرنفل كمضاف طبيعي في ماء الشرب للدجاج اللحم.

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List of abbreviations

Abbreviation Word(s)

FCR	Feed Conversion Ratio
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
DM	Dry Matter
CP	Crud Protein
CF	Crud Fiber
CRD	Completely Randomized Design
GOD	Glucose oxidase
POD	Peroxidase
HB	Haemoglobin
PCV	Packed Cell Volume
WBCs	White Blood Cells
RBCs	Red Blood Cells
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
TP	Total Protein
ALP	Alkaline Phosphatase
CE	Cholesterol Esterase
CO	Cholesterol Oxidase
ANOVA	One Way Analysis of Variance
CG	Control group

CHAPTER ONE

INTRODUCTION

World poultry production has been steadily rising at the rate of 4% annually has become one of the most popular and visible enterprises in Sudan. Profitable poultry industry is always characterized by quick body gain and high egg production with less utilization of feed, according to the records in the Ministry of Animal Resources and Fisheries (2008) Poultry production was estimated to 45.6 million, reported that the modern commercial poultry industry in Sudan was started by the establishment of the Sudanese Kuwaiti Company 1979 and the Arab Sudanese Poultry Company in 1982- 1985 both were centered Khartoum. Then the poultry industry started to grow gradually around Khartoum and other towns in Sudan due to the rising demand for poultry products and the concurrent progress of animal feed industry and the activity of veterinary services.

The food of the population in the tropics is most often of poor protein constituent, both quantitatively and qualitatively, this is because the extreme gap in supply of animal protein. Strong demands for animal protein reflects the increase in population and incomes which considered as main during force behind development of poultry industry. The productivity of poultry have almost tripled in the last 100 years through genetic selection, improved feeding methods, improved housing structure, better disease control, implementation of modern technology and excellent management in addition to processing and improved storage of productions.

The global poultry sector and future trends, and discusses the challenges the sector is facing, with particular emphasis on four areas: food security social

challenges (poverty alleviation and equity), health (animal and human) and environment (natural resources and climate change). Poultry makes a substantial contribution to food security and nutrition, providing energy, protein, and essential micro-nutrients to humans, with short production cycles and the ability to convert a wide range of agro-food by-products and wastes into meat and eggs edible by humans. Poultry is the fastest growing agricultural sub-sector, especially in developing countries. The global poultry sector is expected to continue to grow as demand for meat and eggs is driven by growing populations, rising incomes and urbanisation. In this context, the sector is facing unprecedented challenges. Particularly for small holders and the poor, both in rural and urban areas, poultry is a major asset and key to poverty alleviation, providing income and market participation. Birds can be sold in times of crisis and act as household insurance. But the growing market is essentially benefiting large scale operations and access to market is critical for small holders. However, poultry represent a threat to human health, especially as a vector of infectious diseases and because of its role in antimicrobial resistance. Furthermore, poultry has a significant impact on the environment and is a large consumer of natural resources. While the sector is usually seen as efficient in converting natural resources into edible products, it uses large amounts of land, water and nutrients for the production of feed materials and contributes to climate change, mainly through feed production, air and water pollution. (Anne et al., 2017)

Feed additives are a group of feed ingredients that can cause a desired animal response in a non nutrient role such as pH shift, growth, or metabolic modifier (Hutjens, 1991).

The increasing pressure on the livestock industry to reduce or eliminate feed antibiotics as growth enhancers has initiated new research to find safe and

efficient natural alternatives. This new generation of feed additives includes herbs and their essential oils. (Brenes and Roura, 2010).

Herbs used as feed additives have a significant antibacterial effect, thereby suppress pathogenic micro flora in the gastrointestinal tract of animals and thus reduce mortality during the fattening period, especially in stressful situations (Schone et al.,2006).

Few studies reported that the addition of some aromatic plants and their components in the feed or water ingested by animals, improved live weight gain, feed intake, feed conversion ratio and carcass yield (Criag 1999, Hertrampt. 2001,Tuck., 2002).

Therefore the objective of this study was to evaluate the effects of added graded levels of clove extraction on the performance, some immune response, blood, cholesterol level and some carcass characteristics of broiler chicks.

CHAPTER TWO

LITERATURE REVIEW

2.1. Feed additives:

Animal feed additives are used worldwide for many different reasons. Some of them help to cover the needs of essential nutrients and others to increase growth performance (Giannenas et al., 2005). The use of feed additives is more and more questioned by the consumers. Therefore, the feed industry is highly interested in valuable alternatives which could be accepted by the consumers.

Probiotics, prebiotics, enzymes and highly available minerals as well as herbs can be seen as alternatives (Capcarova et al., 2008). Currently, there is an increasing interest in using herbs and spices in animal nutrition, in order to replace the use of antibiotics and anti coccidials, especially after the ban of antibiotic feed additives within the Europe (Greathed, 2003).

Aromatic plants, also known ; as herbs and spices, have been used since antiquity as folk medicine and as preservatives in food. The best known aromatic plants, such as oregano, rosemary, sage, anise, basil, etc., originate from the Mediterranean area. (Efterpi. 2012).

Cloves are the aromatic dried buds of a tree (*Eugenia caryophyllata* also sometimes *Syzygium aromaticum*) used as a spice in virtually all the world's cuisine. The term 'Clove' is derived from the French word 'Clou' and the English word 'Clout', both meaning 'nail'- from the likeness of the flower bud of the Clove tree to a broad-headed nail. The Clove tree is an evergreen tree, which grows to a height ranging from 8-12m, having large square leaves and sanguine flowers in numerous groups of terminal clusters. The flower buds are at first of a pale color and gradually become green, after

which they develop into a bright red, when they are ready for collecting. Cloves are harvested when 1.5–2 cm long, and consist of a long calyx, terminating in four spreading sepals, and four unopened petals, which form a small ball in the center.(Turner and Jack,2004).

2.2 Clove (*Eugenia caryophyllata*)

2.2.1 Historical background:

During the Han dynasty (207 B.C. to 220 A.D.) those who addressed the Chinese emperor were required to hold cloves in their mouth to mask bad breath. Traditional Chinese physicians have long used the herb to treat indigestion, diarrhea, hernia and ring worm, as well as fungal infection. Indian traditional ayurvedic healers have used clove since ancient times to treat respiratory and digestive tract infections (Kim et al., 1998).

Clove first arrived in Europe around the 4th century A.D. as a highly coveted luxury. The medieval German herbalist used clove as part of an anti-gout mixture. Once clove became easily available in Europe, it was prized as a treatment for indigestion, flatulence, nausea, vomiting and diarrhea. It was also used to treat cough, infertility, warts, wounds and toothache. Early American physicians used clove to treat digestive complaints and added it to bitter herb-medicine preparations to make them more palatable. They were also the first to extract clove oil from the herbal buds. They used it on the gums to relieve toothache. Contemporary herbalists recommend clove for digestive complaints and its oil for toothache (Valero, 2003).

2.2.2 Plant description

Clove *Eugenia caryophyllata* Syn (*Syzygium aromaticum*) or (*Eugenia aromaticum*) is the aromatic dried flower bud of a tree in the family Myrtaceae. It is native to Indonesia and used as a spice in medicine.

all over the world . the name derives from French Cloua nail as the buds vaguely resemble small irregular nails in shape .Cloves are harvested primarily in Indonesia and Madagascar, it is also grown in Zanzibar, India and Srilanka.

At the start of the rainy seasons long greenish buds appear from the extremity of these. The corolla comes which is of a lovely rosy peach color, as the corolla fades the calyx turns yellow, then red . The calyxes with the embryo seed are at this stage beaten from the tree and when dried are the clove of commerce. The flowers have strong refreshing odor. If the seed are allowed to mature, most of the pungency is lost. Each berry has only one seed. The trees fruit usually about (8-9) years after planting . The whole tree is highly aromatic . The spice was introduced to Europe from the fourth to sixth century (Friedrich and Steftem, 1973).

The finest cloves come from Moluccas and Pemba, where the trees grow better than anywhere else, but they are also imported from the east and west Indies, Mauritius and Brazil (Oliver, 1986)

In commerce the varieties are known by the names of the localities in which they are grow .Formerly cloves were often adulterated, but as production increased the price lowered and fraud has decreased . Cloves contain a large amount of essential oil which is much used in medicine when of good quality they are fat oily and dark brown in color, and give out their oil when squeezed with the finger-nail .when pale color and dry , they are inferior quality and yield little oil .Clove stalks are sometimes imported and are said to be stronger and more pungent even than the cloves (Zheng et al, 1992) .

2.2.3 Phytochemistry:

Buds is the part of the clove used .essential oil is also produced from the leaves . The leaves are certainly aromatic enough to make them potentially interesting. The ripe fruits (mother of clove) have only local use . Clove is strongly aromatic and very intensive fragrance fiery and burning taste. The content of essential oil in cloves of good quality may exceed 15%. The oil itself is dominated by eugenol (70 to 85%), eugenolacetate(15%) and alpha and B-caryophyllene (5 to 12%), which together make up 99% of the oil. Cloves contain about 2% of the triter- peneoleonic acid. Volatile oil, gallotannic acid are to crystalline principles .caryohyllin, which is odorless and appears to be a phylosteroleugenin, gum, resin, fiber (Chattopathyay, 2003).

2.2.4 Clove active compound:

The compound responsible for the cloves aroma is eugenol . It is the main component in the essential oil extracted from cloves, comprising 70-85%.Eugenol has pronounced antiseptic and anesthetic properties (Lee and Shibamoto, 2001) .

2.2.5 Uses of clove in medicine:

Clove kills intestinal parasites and exhibits broad antimicrobial properties against fungi and bacteria supporting its traditional use as a treatment for diarrhea, intestinal worms and other digestive oil mends.

Clove are said to have antiseptic and anesthetic properties. Clove and clove oil combat fungal infection, relieve nausea and vomiting, improve digestion, fight intestinal parasites, stimulate uterine contraction, ease arthritis inflammation and stop migraine headache. Patients reported that clove oil may relieve gum and tooth pain and may be useful as a topical antiseptic in

mouth wash (Fetrow and Arial, 1999).The microbiological quality of drinking water is a major public health priority in developing countries. Various parts of the plant i.e. clove, seeds and fruits are used against E. coli in drinking water (Blech et al,1991).Cloves are generally consider safe, although relatively small number of people may be allergic to eugenol (Zheng et al,1992).

Clove is the most stimulating and terminative of all aromatics, given in powder or infusion for nausea, emesis, flatulence, indigestion and used chiefly to assist the action of other medicine . The medicinal properties reside in the volatile oil .The oil must be kept in dark bottles in a cool place .If distilled with water, salt must be added to raise the temperature of boiling and the same cloves must be distilled over and over again to get their full essence . Clove oil as a local irritant stimulates peritonitis. It is a powerful antiseptic; a feeble local anesthetic applied to decayed teeth, and has been used with success as a stimulating expectorant in bronchial troubles. Fresh infusion of clove contains a stringent matter as well as the volatile oil. The infusion and clove water are good vehicles for alkalis and aromatics (Grives, 1995).

2.2.6 Uses of essential oil in medicine:

The antimicrobial properties of essential oils have been known for many centuries. In recent years (1987-2001), along number of essential oils and their constituents have been investigated for their antimicrobial properties against some bacteria and fungi. The classical methods commonly were used for the evaluation of essential oils antibacterial and antifungal activities. The agar diffusion methods and the dilution methods as well as turbid metric and impede metric monitoring of microorganisms growth in the presence of tested essential oils are described. Essential oils are use

against microorganisms in the research are oregano, mint, cinnamon, salvia and clove (Kalemba and Kunica, 2003).

Some essential oils such as clove, eucalyptus, lavender, mint, myrrh and mile folia are used for treatment against inflammatory disease including arthritis, rheumatism and skin allergy and ulcers (Darshan and Doreswamy, 2004).

The antibacterial activity of 11 essential oils from aromatic plants against the food borne pathogen *Bacillus cereus* were examined. The essential oils of clove, nutmeg, mint, oregano, cinnamon, sage and thyme were used against *Bacillus cereus* (Valero, 2003).

2.2.7 Clove safety:

Japanese researchers have discovered that like many spices clove contains antioxidants. Antioxidants help prevent the cell damage that scientists believe eventually causes cancer. On the other hand, in laboratory tests the chemical Eugenol, has been found to be a weak tumor promoter, making clove one of many healing herbs with both pro- and anticancer effects. This point scientists are not sure which way the balance tilts. Until they are anyone with a history of cancer should not use medicinal amounts of clove for otherwise healthy non-pregnant, non-nursing adults powdered clove is considered nontoxic. However, high doses of the oil may cause stomach upset when ingested. When used externally, it may develop a rash (Soltani et al, 2004). Clove and clove oil in medicinal amounts should be consumed only under the supervision of a qualified professional (Soltani et al, 2004).

2.2.8 The effect of adding some other aromatic additives on the performance of broiler chicks:

Mahbubur, et al (2016) studied the effects of *Nigella sativa* seeds (NS) supplementation on meat quality and antioxidants content of chicken meat. There were no significant differences in moisture or crude ash percentage in thigh muscle among groups, but dietary NS powder supplementation resulted in a significant increase in crude protein content and decrease in crude fat content relative to the control group ($p < 0.05$). After 24 h, thigh muscle pH was higher while drip loss, cooking loss and shear force were lower in the NS groups than the control group. Lightness values of thigh muscle color were decreased and redness and yellowness values were increased by NS supplementation. Muscle lipid peroxidation (malondialdehyde) level, which is correlated with significantly higher levels of glutathione peroxidase and superoxide dismutase, were significantly lower ($p < 0.001$) in the NS groups and VE group than the normal group. These results suggest that NS supplementation is effective at improving broiler performance and meat quality by enhancing antioxidant activities and suppressing lipid peroxidation in meat (Mahbubur et al., 2016).

Fenugreek (*Trigonella foenum-graecum L.*) is a medicinal plant having a range of therapeutic, anti-microbial and growth promoting effects. Some researchers studied effects of fenugreek seed powder on growth performance, visceral organ weight, serum cholesterol levels and the nitrogen (N) retention of broiler chicken. The weight gain of the birds that were fed with 4 and 5 % fenugreek was significantly lower ($p < 0.05$) altered by dietary fenugreek. It was concluded that 1 % dietary fenugreek has some growth promoting effects in broiler chicken (Weerasingha et al., 2013).

This study was conducted to determine the use of ca 400 mg/kg Anise oil group with corresponding inclusion levels, and an antibiotic group with 0.1% added antibiotic (Avilamycin). The feed intake was similar in groups ($p>0.05$). The highest ($p< 0.01$) daily live weight gain was observed on the 400 Anise oil group (70.35 g) and followed by Antibiotic group (65.84 g), 100 Anise oil group (62.57g), 200 Anise oil group (62.47 g) and control group (61.30 g). The addition of 400 mg/kg anise oil to the diets was improved daily live weight gain by approximately 15% compared to the control group. This improve was remained 7 % level in antibiotic group. Additionally, the addition of 400 mg/kg anise oil to the diets was improved daily live weight gain by approximately 6.5% compared to the antibiotic group. The addition of 400 mg/kg anise oil to the diets was improved feed conversion ratio by approximately 12 % compared to the control group. This improve was remained 7 % level in antibiotic group. Additionally, the addition of 400 mg/kg anise oil to the diets was improved feed conversion ratio by approximately 6 % compared to the antibiotic group. 7. In conclusion, anise oil could be considered as a potential natural growth promoter for poultry (Naemasa et al., 2015).

A study conducted to investigate the effects of different levels of coriander (*Coriandrum sativum*) seed powder and extract on the performance and carcass characteristics of broiler chickens. It's results suggested that coriander powder in the diet and coriander extract in water could replace synthetic antibiotics and could be regarded as natural feed additives and growth promoters in poultry diets (Muhammed et al.,20130).

Hiefa (2013) studied the effect of dietary anise supplementation on growth performance, some blood parameters and carcass traits of broiler chicks. The results indicated that dietary anise significantly improved total

feed intake, total body weight gain and carcass weight. The highest values of feed intake, body weight gain and final weight were recorded in birds fed anise at level 1%. Moreover, the dietary treatments had no significant effect on total feed conversion ratio FCR and percentage relative weight of liver, heart and abdominal fat. Anise supplementation in broiler chicks diets decreased serum cholesterol, triglyceride and glucose concentration. The best flavor, tenderness, overall performance and returned value were obtained from chicks fed on 1% anise. Accordingly, Anise can be used as phyto-genic feed additive in broiler chicks' diet (Hiefa et al., 2013).

2.2.9 Use cloves in broiler feed:

Heba et al, (2017) studied the effect of dietary clove bud supplementation on growth performance, immune response and antioxidant status of broiler chickens. Clove significantly increased the serum total protein, globulin, cholesterol, levels were significantly decreased. The obtained results stated that BWG, and FCR were improved in Clove supplemented groups respectively, compared to control and other clove supplemented groups. Although, higher doses of clove had no effect on growth performance parameters with enhancement of immunity and antioxidant activities in broiler chickens that concomitantly, provide a healthy broiler's meat with favorable to human consumption.(Heba et al,2017).

Mukhtar (2011) studied the effect of addition different levels of clove oil, as a natural growth promoter, on broiler the performance. Results showed that chicks fed on 600mg/kg clove oil recorded more in feed intake and body weight compared to both control and antibiotic groups ,but this increase was not significant ($p < 0.05$). For feed conversion ratio (FCR) and body weight gain (BWG) chicks fed on diet with antibiotic recorded

numerically the best value. Chicks fed on diets contain clove oil recorded the lowest rate of mortality compared to control group.(Mukhtar et al., 2011).

Hernandez et al, (2010) studied the effect of clove essential oil dietary supplementation on the meat quality of broilers.

He reported that there was no effect of the dietary supplementation with clove essential oil on the lipid content and the fatty acid profile of breast and thigh meat. There was only a small decrease in the percentage of linolenic acid in the thigh meat of the experimental group. Oxidation was not affected by clove essential oil dietary supplementation. Instrumental texture was evaluated in chicken breasts. No differences were observed between the control and experimental group in shear force and firmness, although the total area or work required to complete the shearing of the sample was smaller in the experimental group. Microbiological quality was not affected by experimental treatment. Our results suggest that dietary supplementation with clove essential oil as a growth promoter does not negatively affect chicken meat quality.(Hernandez et al., 2010).

Bestami et al,(2009) studied the effect of different levels of clove extract supplementation in diets on performance and nutrient digestibility in broilers and to determine whether it could be alternative to antibiotics .There were differences in body weight and daily body weight gain at 7. and 21. days measurements in 400 ppm clove extract and antibiotic supplemented groups but differences of the other periods were not statistically important. There was difference in daily feed intake in 400 ppm clove extract and antibiotic supplemented groups at four week of the experiment but differences of the other weeks were not statistically important. The best feed conversion ratio was in 400 ppm clove supplemented group and there were statistically important differences in feed conversion ratio among groups.

The best digestibility degrees were in 400 ppm clove supplemented and antibiotic groups, then 200, 100 and Control groups followed them respectively. Clove extract has the positive effects on performance and digestion process and it is natural and safety feed additive so that 400 ppm supplementation of clove extract to diets can be considered as an alternative natural growth promoter for poultry instead of antibiotics.(Bestami et al., 2009)

CHAPTER THREE

MATERIAL AND METHODS

This experiment was conducted at Animal Research Center, Kuku to evaluate the effect of adding graded levels of clove extract on performance and immune response and some carcass characteristics of broiler chicks. Also the study assessed the economical impact from adding clove to broilers drink water.

This experiment carried out during autumn season (23 January _ 5 march 2019)The ambient temperature ranged from (20 to 37 c) The chemical composition and minerals content of cloves was determined as shown in (table 1)

1. Table1: Chemical composition and minerals in cloves:

Moisture %	D .m%	Ash%	C .p%	C .f%
3	97	5	6.3	13.03
4	96	5	6.7	13.00

Ca	Na	K	Cu	Fe	P	Mg	Mn	Zn
117.5	61.6	111.6	0.4	8.3	1.6	196.8	20.9	1.4
Mg/L	Mg/L	Mg/L	Mg/L	Mg/L	Mg/L	Mg/L	Mg/L	Mg/L

3.2. Preparation of clove extraction:

The cloves were purchased from the local market, cleaned, and amount of 1500 grams of a good quality clove were prepared for the next use. Weighted (0 g/l - 250 g/l - 500 g/l and 750 g/l) of cloves were packing to adding in water through 5 weeks for groups A, B, C and D , respectively. Daily amount of clove was crushed by grinder and put in a glass and add

boiling water to soak for 24 hours at a temperature of 40-50 degrees for 20-30 minutes and leave to be cool down, then add it used in drinking water.

3.3. Experimental Diets:

The broiler chicks were fed the starter diets from up to 21 days to while finisher diet was consumed from 22 days up to the slaughter day. Experimental diets were being adequate in all nutrients matching broiler chicks requirements (NRC, 1994) was offered to the chicks as finisher diets. Ration ingredients were sorghum, groundnut cake, concentrate methionine and lysine, salt, limestone. The experimental diets were fed for 42 days as shown in table 2

Table 2. Percent inclusion rates (as fed basis) and calculated analysis (dry matter basis) composition of experimental diets.

Ingredient diets	Starter 7-21 days	Finisher 22-42 days
Sorghum	63	70
GNC	30	24.2
Limestone	0.75	0.1
DCP	0.6	0.15
Lysine	0.2	0.1
Methionine	0.15	0.15
Concentrate	5	5
Salt	0.2	0.2
Mycotoxin binder	0.1	0.1

Calculated Analysis:

Items	Starter	Finisher
Energy Mg/Kg	12.97	13.39
Protein	23	19
Ca	1.1	1
P	0.5	0.5
Ly	1.12	1.12

*Concentration composition:

Ingredients broiler starter: Metabolisable energy 1800 kcal/kg, crude protein 35, crude fat 1.09, crude fiber 0.10, Ca 13.20, P 9, Ly 22, Meth 9

Ingredients broiler finisher: Metabolisable energy, crude protein, crude fat, crude fiber,

3.4. Housing:

Semi closed wire mesh _side poultry house was used. That was designed up to 5m in width and 15m in length with height 3m from the foundation to roof line. The house was constructed from corrugated metal sheets roof with low masonry walls set on a concrete floor and wire mesh on the upper part of the walls providing good ventilation, with solid brick western eastern wall. The house was contained 20 pens partitioned with wire mesh 1m of each. Feed and water were provided ad libitum consumption during whole experimental period.

Light was provided approximately 24 hours light (natural / artificial) for increasing feeding time 60-100 watt bulbs were used according to requirement. The density of birds was 10 birds/m².

3.5.Experimental Birds :

A total number of (200 birds _day old) commercial unsexed broiler of Cobb500 bird were purchased from (Mico poultry company)and transported to the Animal Production Research Center. The live body weight of chick upon receipt was 45g.

The chicks were adopted to the premises and feed over 7days before the start of the experiment .At the end of adaptation period, all chicks were weighted with an average initial weight of 135g.The chicks were then allotted randomly in to four treatments groups (A,B,C and D) in Completely Randomized Design (CRD), each group was divided in five replicates, each of 10 chicks .Ground brooding rearing system was adopted for 5weeks experimental period . chicks were bought vaccinated against (IB)and New Castle diseases (ND) at 3days of age through spraying ,Gumboro disease at 14days of age through drinking water , retard Gumboro disease at 21days of age through drinking water and Newcastle disease at 28days of age Soluble multi vitamin compounds (supper san) were given during after vaccinated.

3.5.1 Internal organs weight:

At the end of the experiment (42 days), birds were weighted and fasted overnight (except for water). Three birds from each replicate were randomly selected and slaughtered without stunning, then scalded, manually plucked, washed and allowed to drain on wooden table. Evisceration was performed by a ventral cut and visceral as well as thoracic organs were removed. Liver, Heart and gizzard individually weighted.

3.6 Broiler performance parameter:

3.6.1 Body weight:

Live body weight was measured at the beginning of the experiment, and then it was repeated on weekly basis at the end of each week at the same time.

3.6.2 Body weight gain:

Body weight gain was calculated by subtraction live body weight at the beginning of each week from live body weight at the end of the same week.

3.6.3 Feed intake:

Feed intake was calculated by subtracting the remaining from offered feed at end of week from the weight of feed given at the beginning of the same week.

3.6.4 Feed conversion ratio:

It is the amount of feed (g) needed to produce (g) of meat, as it was calculated by dividing the feed intake (g) \ gain (g).

3.6.5 Mortality rate:

The rate of mortality was determined by calculating the ratio between the numbers of the dying bird and initial total number of birds multiplied by 100 of each week until week 6.

3.7 Haematological tests:

3.7.1 Blood samples collection:

Blood samples were collected from 60 chicks in the morning (around 9:00 AM). Five milliliters of whole blood was collected aseptically from the jugular vein via jugular vein puncture into Vacutainer tubes.

Blood was divided into two portions. The whole blood was kept in tubes with anticoagulant (EDTA) and was utilized for determination of haematological indices.

The other portion of the blood was kept in plain tube without anticoagulant. Serum was obtained by allowing the blood to clot in room temperature for at least 30 minutes, and the tube were centrifuged at 3000rpm for 5min to separate serum from cells. Serum samples were harvested in special epindoorf containers and stored at -20c until analyzed . Biochemical analysis was performed on serum utilizing spectrophotometer. Serum was analyzed for aspartate aminotransferase (AST), alkaline phosphatase (ALT), cholesterol, total protein, albumin, glucose and golobulin.

3.7.2 Blood constituents analysis:

Blood samples were examined for haemoglobin concentration (HB), packed cell volume (PCV), red blood cells (RBCs) and white blood cells (WBCs) differential count. All haematological indices were investigated using standard methods with automated Haematological Analyzer (Sysmex KX-21).

These techniques were performed according to Automated Hematology Analyzer (Sysmex KX-21, 1999) manual for in vitro diagnosis used in Pharmacology laboratories. The Sysmex KX-21 processes approximately 60 samples /hour and was displayed on the LCD screen, for measure of WBCs, RBCs count along with data of other parameters.

3.7.3Blood and serum profiles:

Blood samples with drawn from jugular veins were analyzed for a complete hemogram, HB concentration, PCV, RBCs and WBCs counts.

Serum prepared from the same sample with drawn was analyzed for concentration of metabolites total protein, cholesterol, glucose, enzyme activities GOT, GPT, T.protein, Albumen and Golobulen.

3.7.4 Aspartate aminotransferase (AST)determination method:

This enzyme is also known as aspartate transaminase, L-Aspartate: 2-oxoglutarate aminotransferase, glutamate oxaloacetate transaminase (GOT,E.C2.6.1.1). Serum AST activity was measured by a spectrophotometric method using commercial kits (biosystems S.A; Spain).

3.7.5 Alkaline phosphatase (ALP) determination method:

The kinetic method present is optimized in accordance with (D.G.K.C) the principle is catalyzes the hydrolysis of p-nitrophenol phosphate in p-nitrophenol and phosphate, in alkaline medium .

3.7.6 Glucose:

Glucose in the sample originates, by means of the coupled reactions, a colored complex that can be measured by spectrophotometer.

3.7.7 Total Protein:

Protein in the sample reacts with copper (II) ion in alkaline medium forming a colored a complex that can be measured by spectrophotometer.

3.7.8 Albumin:

Albumin in the sample reacts with bromocresol green in acid medium forming a colored complex that can be measured by spectrophotometer.

3.7.9 Cholesterol:

Free and esterified cholesterol in the sample originates, by means of the coupled reactions, a colored complex that can be measured by spectrophotometer.

3.8. Slaughter Procedure:

At the end day of the period, the birds prevented from feed all the night and weighted individually before slaughter severing the right and left carotid with jugular vessels, trachea and esophagus. After complete bleeding birds scalded in hot water, hand-plucked and washed. The head removed closed to skull, also feet with shanks removed at the hock joint.

Evisceration accomplished by make the opening in a posterior ventral. Hot carcass, giblets (heart, liver and gizzard), were weighted and recorded.

3.9 Statistical Analysis

The experimental design was completely randomized. Data generated from the experiment were subjected to analysis of variance(One –way-ANOVA) and the mean were tested for significance by least significant (LSD) using the statistical package of social science (SPSS) (Version 16) Gomez and Gomez .,(1984) computer program.(Obi, 1990).

CHAPTER FOUR

RESULTS

Response of broiler chicks to clove extract

4.1. performance

No significant differences in weekly body weight gain were observed among all treatment groups during the 1, 2, 3 and 4 week of the experiment. However, a significant differences was recorded among experimental groups ($P < 0.05$) at week five.

Table 3. Weekly Wt gain (g/bird) of studied broilers chickens

Treatments Weeks	Treatment				Significant
	0	250	500	750	
Week 1	323.00±11.13	302.90±46.35	311.10±13.20	313.30±17.1 6	NS
Week 2	653.70±39.32	668.20±30.06	642.00±25.78	653.80±28.1 3	NS
Week 3	1060.60±46.z 77	1109.30±43.85	1104.00±47.31	1110.00±33. 57	NS
Week 4	1562.70±81.9 3	1640.20±91.49	1617.60±84.83	1655.90±81. 10	NS
Week 5	2062.00±100. 35	2164.10±103.88	2070.20±103.69	2115.40±10 4.08	*

a, b, c :mean within the same row followed by different superscripts are significantly ($P < 0.05$) different

NS: No significant different at ($p < 0.05$) og/l: control group

250g/l: clove extract used 250g/l 500g/l: clove extract used 500g/l

750g/l: clove extract used 750g/l *: significant different ($p < 0.05$)

Table (4) showed the weekly feed intake g/bird. The results recorded No significant differences among groups during the 1, 2, 3, and 4 week But at week five significant differences ($P < 0.5$) was observed among experimental groups.

Table4. Weekly feed intake (g/bird) of studied broiler chickens

Treatments Weeks	Treatment				Significant
	0	250	500	750	
Week1	352.18±23.27	348.30±20.47	347.60±28.28	345.70±18.58	NS
Week2	541.81±18.33	544.70±9.09	552.00±39.04	534.22±11.50	NS
Week3	748.93±31.90	790.60±48.06	777.50±13.10	773.90±17.24	NS
Week4	988.43±61.25	1022.30±44.57	982.60±62.34	1023.50±50.90	NS
Week5	925.36±37.25	986.40±45.44	925.00±63.90	979.48±38.15	*

a, b, c :mean within the same row followed by different superscripts are significantly ($P < 0.05$) different

NS: No significant different at ($p < 0.05$) 0g/l: control group

250g/l: clove extract used 250g/l 500g/l: clove extract used 500g/l

750g/l: clove extract used 750g/l *: significant different ($p < 0.05$)

The supplementation of clove in drinking water revealed into no significant differences level ($p < 0.5\%$) among experimental groups at week 1, 2, 3 and 4 week groups during the 1, 2, 3 and 4 week except of week 5 significant differences was observed.

Table (6) showed the effect of added clove to drink water feed conversion ratio. Results shown no significant differences in feed conversion ratio between all treatments group during the 1, 2, 3, 4 and 5 week.

Table 6. Weekly FCR (g/bird) of studied broiler chickens

Treatments Weeks	Treatment				Significant
	0	250	500	750	
Week1	1.84±0.11	1.95±0.26	1.95±0.14	1.92±0.17	NS
Week2	1.65±0.18	1.57±0.12	1.67±0.15	1.57±0.07	NS
Week3	1.78±0.11	1.79±0.06	1.70±0.14	1.70±0.06	NS
Week4	1.86±0.06	1.80±0.08	1.86±0.10	1.80±0.07	NS
Week5	1.86±0.09	1.88±0.08	1.97±0.06	2.14±0.19	NS

a, b, c :mean within the same row followed by different superscripts are significantly ($P < 0.05$) different

NS: No significant different at ($p < 0.05$) 0g/l: control group

250g/l: clove extract used 250g/l 500g/l: clove extract used 500g/l

750g/l: clove extract used 750g/l

No significant differences in weekly body weight gain in study of experimental all period between all treatment groups during the 1, 2, 3,4 and5 week of the experiment.

Table 7. Weekly Wt(g/bird) of studied broiler chickens

Treatments Weeks	Treatment				Significant
	0	250	500	750	
Week1	3521.8±232.68	3483±204.71	3476±282.80	3457±185.79	NS
Week2	5311±328.22	5447±90.93	5263.3±168.08	5342.2±114.99	NS
Week3	7332±277.68	7720±240.72	7657±212.26	7739±172.38	NS
Week4	9671±430.65	10362±318.30	9826±623.35	10235±508.95	NS
Week5	9253.6±372.47	9864±454.40	9250±639.00	9600±602.01	NS

a, b, c :mean within the same row followed by different superscripts are significantly (P<0.05) different

NS: No significant different at (p< 0.05) og/l:control group

250g/l:clove extract used 250g/l 500g/l: clove extract used 500g/l

750g/l: clove extract used 750g/l

Table (7) showed the overall performance of broilers drink clove extract with water. The results showed highly significant differences ($P<0.01$) for feed intake, weight gain and final body weight. On the other hand, feed conversion ratio (FCR) and mortality, birds had not affected by adding clove. Birds drinking concentration of clove 0.5% were showed a higher values of most performance parameters followed by others concentration which recorded high value than control groups.

Table7.Effect of clove extract on overall performance of broiler chicks.

Treatments weeks	Treatment				Significant
	0	250	500	750	
Number of chicks	50	50	50	50	-
Experimental period	35	35	35	35	-
Initial Wt	131.8±26.59	133.1±16.73	132.5±21.50	132.1±18.50	-
Feed intake(g/bird/day)	101.62±4.9	105.49±4.7	102.42±5.9	104.48±3.9	*
Weight gain(g/bird/day)	58.9±2.8	61.83±2.9	59.14±2.9	60.44±2.9	*
Feed intake (g/bird/week)	3556.7±172	3692.3±167.63	3584.7±206.66	3656.8±136.37	**
Weight gain (g/bird/week)	2062±100.35	2164.10±103.88	2070.20±103.69	2115.40±104.08	**
F C R (g feed/g gain)	1.72±1.7	1.70±1.6	1.73±1.9	1.72±1.3	NS
Final body weight(g/bird)	2090.30±111.19	2204.80±62.48	2159±64.84	2169.30±54.70	**
Mortality%	0.1%	0.1%	0.1%	0.1%	NS
Profitability Ratio	0.90	0.95	0.98	1	**

a, b, c :mean within the same row followed by different superscripts are significantly (P<0.05) different

NS: No significant different at (p< 0.05) og/l:control gr oup

250g/l:clove extract used 250g/l 500g/l: clove extract used 500g/l

750g/l: clove extract used 750g/l *: significant different (p< 0.05)

** :highly significant (P<0.01)

4.2. Serum metabolites

The serum metabolites values of broiler chicks clove extract shown in Table (8).The parameters of blood biochemistry (PCV, HB, WBCs and RBCs) were not affected by addition of clove to drinking water. Which the glucose level was decreased (P<0.05) than its level in control group.

Table 8.The effect of Clove extract on blood Biochemistry of broiler chicks

Treatments weeks	Treatment				Significant
	0	250	500	750	
PCV	31.61± 2.12	32.53± 2.21	31.47± 2.59	31.00± 2.07	NS
HB	13.83± 0.61	13.73± 0.64	13.47± 0.63	13.57±0. 52	NS
WBCs	6.64± 0.53	6.70± 0.63	6.79± 0.49	6.48± 0.44	NS
RBCs	6.35± 0.69	6.62± 0.57	6.33± 0.62	6.45± 0.43	NS
Glucose	47.06± 3.02	45.21± 1.08	45.13± 0.84	45.33± 1.32	*
Globulin	0.95± 0.81	1.82± 0.59	2.33± 0.59	1.53± 0.52	**

a, b, c :mean within the same row followed by different superscripts are significantly ($P < 0.05$) different

NS: No significant different at ($p < 0.05$) og/l:control group

250g/l: clove extract used 250g/l 500g/l: clove extract used 500g/l

750g/l: clove extract used 750g/l *: significant different ($p < 0.05$)

** : highly significant ($P < 0.01$)

Table (9) showed the effect of clove extraction on serum enzymes and cholesterol level. A highly significant differences ($P < 0.01$) were reported among experimental group for Got, Gpt, cholesterol level and ALB.

Table 9. The effect of Clove extract on serum enzymes and serum metabolites values of broiler chicks

Treatments weeks	Treatment				Significant
	0	250	500	750	
Got	59.64±5.92	69.05±5.66	71.26±4.29	70.61±5.85	**
Gpt	19.75±5.69	15.11±2.50	13.77±5.46	12.26±4.04	**
Cholesterol	63.95±1.55	59.41±1.45	57.91±1.49	55.05±3.52	**
T. P	4.64±0.57	4.89±0.57	5.41±0.54	5.13±0.42	*
ALB	3.88±0.93	3.06±0.10	3.05±0.22	3.59±0.36	**

a, b, c :mean within the same row followed by different superscripts are significantly ($P < 0.05$) different

NS: No significant different at ($p < 0.05$) og/l:control gr oup

250g/l: clove extract used 250g/l 500g/l: clove extract used 500g/l

750g/l: clove extract used 750g/l *: significant different ($p < 0.05$)

** : highly significant ($P < 0.01$)

4.3. Carcass and measurement

4.3.1 Carcass dressing

The results indicated no significant differences ($p>0.05$) among treatment groups in carcass dressing as shown in Table (10) Mean values of all group are similar, but group (0.5%) recorded the highest mean values in all traits.

Table 10. The effect of Clove extract on the performance and dressing percentages of broiler chicks

Treatment Week	Treatment				Significant
	0	250	500	750	
Initial Wt	131.8±26.59	133.1 ± 16.73	132.5±21.50	132.1±18.5 0	NS
Final Wt	2090.30±111. 19	2204.80±62. 48	2159.00±64.8 4	2169.30±5 4.70	**
Hot Carcass	1466.00±83.3 9	1514.00±55. 97	1489.00±54.5 8	1506.70±7 2.84	*
Dressing %	70.31	70.67	70.42	70.45	NS

a, b, c :mean within the same row followed by different superscripts are significantly (P<0.05) different

NS: No significant different at ($p < 0.05$)

og/l:control group

250g/l: clove extract used 250g/l 500g/l: clove extract used 500g/l

750g/l: clove extract used 750g/l

*: significant different ($p < 0.05$)

** :highly significant (P<0.01)

4.3.2 Non carcass yield

The effect of clove extract on the percent values of the giblets (liver, gizzard and heart)of broiler chicks shown in Table(10).The result showed that the treatment effect on the percent of all giblet part was not significant ($p > 0.05$).

Table 10.The effect of Clove extract on the percent of giblets (liver, gizzard and heart) of broiler chicks

Treatments weeks	Treatment				Significant
	0	250	500	750	
Heart 29	11.33±2.	10.67±1.76	10.67±1.76	10.00±0.00	NS
Gizzard 41	43.00±8.	43.67±5.50	40.00±4.63	43.33±5.56	NS
Liver 16	46.33±5.	46.00±4.71	43.00±6.21	44.00±4.71	NS

a, b, c :mean within the same row followed by different superscripts are significantly ($P < 0.05$) different

NS: No significant different at ($p < 0.05$) og/l:control group

250g/l:clove extract used 250g/l 500g/l: clove extract used 500g/l

750g/l: clove extract used 750g/l

4.4. Economic appraisal

The total cost, returns and profitability ratio per head of broiler chicks given graded levels of clove extract for 6 weeks are shown in Table (12). Chicks purchase, feed, management cost values (SDG) were the major inputs considered. The total selling values of meat of the total revenues were obtained. Profitability ratio (1) of test group (D) the clove extract concentration 750g/l was the significant highest value among the test groups.

Table 12. The effect of Clove extract on economic appraisal/bird (SDG)

Items	Treatment			
	0	250	500	750
		Cost		
Chick purchase	20	20	20	20
Total feed cost	70	70	70	70
Management	10	10	10	10
Total cost of production	100	100	100	100
		Revenue		
Live weight	2090.30	2204.80	2159.00	2169.30
Dressing percent	70.42	70.67	70.31	70.45
Average weight	0.67	0.68	0.69	0.69
Price/kg of bird	115	115	115	115
Total Revenue	67.48	68.81	69.30	69.21
		Profit		
Total Revenue	67.48	68.81	69.30	69.21
Total cost of production	100	100	100	100
Total Profit	22	31.19	30.70	30.79
Profitability Ratio	0.90	0.95	0.98	1.00

Total cost calculated according to prices during March 2019

A current (March 2019) price of meat 115 (SDG) Kg

CHAPTER FIVE

DISCUSSION

Natural medicinal products originating from herbs, species and their extract products have been used as feed additives in poultry production (Hashemi and Davoodi, 2010; Khan et al.,2012).

The current study was conducted to investigate the influence of using clove extract added to drinking water on broiler chicks on the overall performance, blood chemistry and some hematological parameters. The results showed that given broiler chickens with different concentration levels of clove extract in water they would numerically consumed more feed than control group. Added clove extract to the drink water of broilers had highly significant ($p < 0.01$) increased feed intake, weight gain, FCR and profitability ratio of broilers chicks than that in control group. This result was in disagree with Heba et al.,(2017) and Weerasing.,(2013) they reported that no significant on feed conversion ratio (FCR) of broilers was not affected experimental treatments compared with groups, this results was agree with finding Heba et al.,(2017).The clove extract could act to antibiotics. This can results in a higher efficiency in the feed utilization, and it can leads to a higher weight gain and better feed efficiency. According to improvement broiler performance by clove extract probably is due to antibacterial and antifungal effects originated and improve healthy level. It may be reason that spices and herbs will positively affect food digestion. Body weight and other organs can increase by improving the nutrient absorption. Also, as itmentioned the using of clove extract resulted in

increasing of feed intake. The positive influence in BW, FCR and feed intake of broilers offered clove extract had best values compared with broiler without any clove Bestami (2009).

The weight of internal viscera (liver, heart and gizzard) slaughter indicated that heart and liver size decrease when added clove extract compared with control. This results was in line with that reported by Gamal eldin et al, (2021).They stated that the increment in weight of carcass compounds (liver, heart and gizzard) was observed in low levels of clove.

Blood chemistry parameters which taken in the present study was not affected by the clove extract. Regarding result of the current study on the hematological parameters (Red Blood Cells, White Blood Cells, Packed Cell Volume and Haemoglobin)were not influenced by the clove extract except glucose reduced and globulin increased, gives immunity against diseases and thus the safety and health of the herd. Clove significantly increased the total protein. This result disagree with findings by saud, et al (2019) he reported that high levels of clove (4%, 5%, and6%) supplementation resulted in reduced total serum protein and albumin. This may be due to the high levels of clove added to the diet than our low clove extraction added to drinking water in this study.Cholesterol was significantly decreased. The normal cholesterol level in poultry. While the value recorded in this study was affect of serum (Got and Gpt) of all groups were significantly affect Got increased and Gpt decreased.

This substance is beneficial for broiler and human health.

Conclusion and Recommendation

Conclusions:

Based on the result of the study the following conclusions can be withdrawn: the addition of clove extract to drinking water of chicks was significantly increasing body weight gain and feed intake, However feed conversion ratio was not improved, the addition of (0.5%) concentration of clove extract give the best performance compared to other groups.

Recommendations:

The result obtained from this study demonstrated that there are great different between 0.5%, 1% and 1.5% clove extract concentration in overall performance of broiler chicks compared to control group.

Clove can be used in broiler rations at level 0.5% to give positive response in broiler performance and had effect on the serum level, cholesterol and immune response from previous studies and above results obtained in this research, clove can be considered as medicinal plant.

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