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Effect of adding water extract of *Syzigium aromaticum* to drinking water in the microbial characteristics of broiler intestines

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Abstract:
This study was aim to determine the microbial content in the intestines of broilers treated with water extract of carnation flowers for drinking water, represented by the logarithmic numbers of intestinal and colon bacteria, 180 broiler chicks Ross308 were used, distributed randomly into four treatments, each treatment consisted of 45 chicks, with three replicates per treatment and 15 chicks per replicate. The experimental treatments comprised of four groups: T1, which served as the control treatment without any addition, T2, T3, and T4: adding water extract of carnation flowers at a rate of 200, 300 and 400 ml / liter of drinking water, respectively. Measurements were taken at the fifth week. According to the results, the addition treatments showed a significant decrease (P≤0.05) in the numbers of total aerobic bacteria and coliform bacteria when compared to the control treatment, the percentage of the decrease with the increase in the percentage of addition of the extract, with a significant increase (P≤0.05) in the numbers of anaerobic bacteria Lactobacilli bacteria for the addition treatments compared to the control treatment, the percentage of rise increases with the increase in the percentage of addition of the extract compared to the control treatment.

Keywords: microbial characteristics, broiler intestiens, Syzigium aromaticum.

Introduction

Carnation (*Syzygyum aromaticum*) is an evergreen tree with a pyramidal shape, 15 meters high. Myrtaceae carnation is a dried flower bud, the original homeland of carnation is the Molaca Islands, Indonesia and the southern Philippines, there was more than one name for carnation in the Arab world. It was known as the nail for the similarity between it and the nail, it was also called Oud al-Nuwar and has a strong aromatic smell (Hussein, 2015).

It is one of the important medicinal plants as it is used either directly or by one of the plant derivatives (extract or essential oil). The most important characteristic of clove medicinally is that it contains biologically active substances, which volatile compounds are secondary metabolites, synthesized in plants in response to stimulation of harmful chemicals produced by pathogenic microbes or environmental cues (Reichling, 2010).

An important role in promoting plant health and defense (Stamp, 2003). It contains phenols, aldehydes and terpenes, capable of disrupting bacterial function (Pasqua *et al*., 2007). The most important of these molecules are (eugenol, cinnamaldehyde and carvacrol) (Juven *et al*., 1994; Jay and Rivers, 2007). Eugenol (4 allyl-2-methoxyphenol) (a phenolic compound), the main ingredient found in carnations, European Commission registered antimicrobial, to use it as a flavoring ingredient without posing any health risk to the consumer (Burt, 2004).

It has been shown to have activity against Gram-positive and Gram-negative bacteria (Qiu *et al*., 2010). Antifungal and antiviral activities (Chami *et al*., 2005; Astani *et al*., 2009). Oxidative stress,

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inflammation, and spasm (Gill and Holley, 2004; Clinical and Laboratory, 2005; Mohammed and Al-Bayati , 2009).

The importance of the active ingredients of this medicinal plant, it can be used as an alternative to antibiotics in poultry feed, it is also used in a wide range of aromatic plants, as a natural alternative to chemical pesticides in controlling fungal and bacterial plant pathogens (Montes and Prados, 2006; Mohamed and Ali, 2015).

This research aims to use different levels of aqueous extract of carnation flowers in drinking water on some microbial groups of the alimentary canal (duodenum) and caecum of broilers, the microbial species and groups in the gut important, called intestinal flora in the events of microbial balance, reflected in the general health of chickens.

Materials and Methods

This experiment was carried out in one of the private poultry farms, from 15/9/2021 to 19/10/2021. A total of 180 unsexed, one day, 40 g Ross 308 broiler chicks were used, were randomly distributed to four experimental treatments, according to a completely randomized design, each treatment included three replicates (15 chicks/replicate). The treatments were as follows:

T1: (control).
T2: Add aqueous extract of carnation flowers at a rate of 200 ml / liter of drinking water.
T3: Add aqueous extract of carnation flowers at a rate of 300 ml / liter of drinking water.
T4: Add aqueous extract of carnation flowers at a rate of 400 ml / liter of drinking water.

The carnations utilized in the experiment were purchased from local markets. The water extract of carnation flowers was prepared using a modified method (Hernandez *et al*., 2004). In this method, dry carnation flower powder was mixed with distilled water in a ratio of 1 g to 2 ml of distilled water, using an electric mixer. The resulting solution was left to settle for 24 hours at room temperature, and then filtered through multiple layers of sterile medical gauze. The extract obtained was added to drinking water at the three levels mentioned (200, 300, 400 ml extract/liter of drinking water). Water was provided to the treatments for each treatment according to the level of addition until the end of the experiment.

Table (1): Chemical composition of clove flower powder.

Contents	Dry weight basis (%)
Moisture	30.18
Crude protein	6.17
Crude fat	5.22
Soluble sugars	31.47
Crude fiber	14.08
Ash	4.93

The birds were fed two types of diets, starter diet from 1-21 days, the second diet was a grower diet from 22-35 days, were calculated according to NRC (1994) recommendations (Table 2), when the birds reach the age of 5 weeks, one bird from each replicate (3 birds/treatment) was slaughtered under sterilization conditions. Samples were taken from the contents of the small intestine (duodenum) and cecum. Microbial tests were done, included estimating the total number of aerobic bacteria using Nutrient Agar culture media, estimation of the total number of coliform bacteria using MacConKey Agar culture media, estimation of the total number of Lactobacillus acidophilus bacteria using Agar MRS culture media, Pour Plate Count method, after making the required decimal dilutions, the number of microbial aggregates was estimated from each (1 ml) of the contents of the small intestine and cecum

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Table (2): Diets nutritional and chemical compositions.

Ingredient %	Starter (1-21 days)	Grower (22-35 days)
Yellow corn	42.50	46.50
Wheat	18.00	18.00
Soybean meal	32.00	27.50
***Protein concentration**	4.00	4.00
** **Premix**	1.00	1.00
Limestone	2.00	1.50
Plant oil	0.50	1.50
Total	100.00	100.00
Calculated chemical composition		
Crude protein (%)	23.10	21.30
Metabolize energy (kilo calorie/ kg diet)	2954	3072
Calorie: protein ratio	127.87	144.22
Crude fiber (%)	3.82	3.85
Calcium (%)	0.92	0.86
Phosphorus (%)	0.48	0.45
Methionine (%)	0.55	0.51
Lysine (%)	1.32	1.24
Methionine + Cystine (%)	0.90	0.84

*The protein concentrate for broiler feeding with a chemical composition of 40% crude protein, 5% crude fat, 2.20% crude fiber, 7.10% moisture, 28.30% crude ash, 4.20% calcium, 4.65% phosphorus, and 2107 kcal. g-1 metabolizable energy. Additionally, the company offers premixes with a chemical composition of 10% crude protein, 2.1% crude fat, 0.34% crude fiber, 2.66% moisture, 51.02% crude ash, 20.08% calcium, 10.83% phosphorus, and 753.82 kcal/kg metabolizable energy.

Statistical analysis

A Completely Randomized Design (CRD) was used to analyze the data, and the significance of the differences between the means was determined using Duncan's multiple range test (Duncan, 1955) with a significance level of 0.05 and 0.01. The analysis was conducted using SAS statistical software (SAS, 2001).

Results and discussion

Table 3 presents the impact of utilizing aqueous extract of carnation flowers on the logarithmic counts of total aerobic bacteria, coliforms, and lactobacilli in the duodenal and caecal contents of broilers. The results indicate a significant reduction ($P \leq 0.05$) in the logarithmic counts of total aerobic bacteria and coliforms in both the duodenum and caecum regions when the aqueous extract was added, compared to the control treatment. Additionally, there was a significant decrease ($P \leq 0.05$) in treatment T4 compared to treatments T3 and T2, which also exhibited significant reductions, respectively, when compared to the control treatment.

The table shows a significant increase ($P \leq 0.05$) in the logarithmic numbers of Lactobacilli bacteria for T4 treatment in the duodenum and caecum regions compared with the rest of the treatments. Also, a

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significant increase (P≤0.05) was observed for treatments T3 and T2 compared to the control treatment.

Table (3) Effect of using water extract of clove flowers on the logarithmic numbers of total aerobic bacteria, Colliforms and Lactobacilli (gr/ cfu) of the contents of the duodenum and caecum for broilers (mean ± standard error).

Treatments	duodenum			Caecum		
	total aerobic bacteria	Colliforms	Lactobacilli	total aerobic bacteria	Colliforms	Lactobacilli
T1	± 5.33 0.06a	± 10.49 0.10a	± 3.72 0.04d	± 4.15 0.04a	± 7.59 0.06a	± 2.67 0.02d
T2	± 5.04 0.03b	± 10.06 0.08b	± 4.12 0.03c	± 3.32 0.03b	± 7.25 0.05b	± 3.11 0.03c
T3	± 4.65 0.05c	± 9.85 0.11c	± 4.32 0.04b	± 2.37 0.03c	± 7.03 0.05c	± 3.34 0.03b
T4	± 4.46 0.05d	± 9.64 0.06 d	± 4.55 0.03a	± 2.15 0.04d	± 6.83 0.06d	± 3.56 0.02a
Sig.	*	*	*	*	*	*

The experimental treatments were as follows: T1 represented the control treatment without any addition, while T2, T3, and T4 represented the addition of the aqueous extract of clove flowers at rates of 200 ml, 300 ml, and 400 ml per liter of drinking water, respectively. The statistical analysis showed that there were significant differences (P≤0.05) between the groups in each column, as denoted by the different letters within the column.

The use of carnation flower extract in the treatments resulted in the best outcomes, as it effectively decreased the number of harmful bacteria (total aerobic bacteria and coliforms) and increased the number of beneficial bacteria (anaerobic bacteria) such as Lactobacillus acidophilus and lactobacilli bacteria. This plant extract exhibited a positive impact in reducing the number of harmful bacteria. The reasons for this may be due to the fact that the extract contains some chemicals, that act as antimicrobials, which had a significant role in reducing the total numbers of aerobic bacteria and colon bacteria in the duodenum and cecum, agreed with what was mentioned Dorman and Deans (2000); Friedman *et al*. (2002), as they showed that cinnamaldehyde, thymol and carvacrol are very active molecules against a wide range of bacterial species, he mentioned (Bowers and James, 2000). The inhibitory effect of microbes may be due to the presence of alkaloids and tannins with antiseptic properties in the leaves extract such as pinene, phenylene and eugenol, which have positive effects against pathogenic microbes.

Plant compounds cause damage to the cell membrane, loss of energy production, enzyme imbalance, and leakage of the internal contents of the cell, lead to dysfunction of cellular organs and cell death (Upadhyay *et al*., 2014). Recent research revealed that plant compounds affect gram-positive and gram-negative bacteria (Upadhyay *et al*., 2012 and 2013; Mooyottu *et al*., 2014), by modulating gene transcription (Qiu *et al*., 2010). Protein expression and sensory expression (Koh *et al*., 2013; Ahmad *et al*., 2015). Gene modification and cell transcription, the plant compounds not only work by lowering the receptors for toxins, but by modifying critical toxicity genes, expression of the host sensor, which leads to the reduction of toxins.

According to Fazaa (2013), the extract from carnation flowers has a significant effect on bacteria sensitivity. This could be due to several factors, such as the chemical affinity of the extract to interact with cell components, The bacterial cell wall may have specific receptors or transport vectors that allow the extract molecules to enter the cell and inhibit the activity of co-enzymes and other biologically active molecules. These results are in agreement with the findings of Abbas, (2010).

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Carnation flowers contain high levels of eugenol (70-90%) which may play a crucial role in inhibiting the number of bacteria in the intestines and cecum, as reported by Scherer *et al*. (2014). Eugenol and capsid have been found to have significant antimicrobial activity, and the structure of the eugenol compound can serve as a basis for the development of antimicrobial agents, as demonstrated by Qiu *et al*. (2010). Eugenol works by overlapping one or more regulatory genes in bacteria, affecting transcription of genes and leading to inhibition and suppression of genetic transcription, which in turn affects bacterial reproduction and prevents the secretion of anthrotoxin from bacteria. This helps to reduce negative effects on public health. The inhibitory effects of eugenol have also been confirmed in other studies such as Blaszyk and Holley (1998) and Ben Arfa *et al*. (2006), which reported the inhibition of E. coli growth and other bacteria by eugenol. An increase in the number of beneficial bacteria (anaerobic bacteria), represented by Lactobacilli bacteria, could be due to the antimicrobial effect of the active substances present in the essential oils of aromatic plants, the most important of which is eugenol that is selective for some harmful microbes (Ouwehand *et al*., 2010). Thymol, eugenol and cinnamaldehyde caused a decrease in the number of coliform bacteria, but the beneficial bacteria were not affected to the same degree, agreed with Michiels *et al*., (2009), they showed that eugenol has a positive choice towards lactic acid bacteria, led to an increase in the number of lactobacilli bacteria in the intestines of broilers treated with carnation. Tiihonen *et al*. (2010) conducted a study that demonstrated the potential of aromatic plants to improve gut microbiology and activity, which can lead to improved growth due to the active substances that stimulate increased mucus secretion in the small intestine. This reduces the adhesion of pathogens to the intestinal epithelium, as noted by Jamroz *et al*.(2006) .

The results of the current study clearly indicate the effectiveness of using an aqueous extract of carnation flowers to promote microbial balance and maintain it by controlling beneficial bacteria, such as lactobacilli and *Lactobacillus acidophilus*, while limiting the growth of harmful bacteria through competitive exclusion of coliform bacteria. The extract caused a rapid decrease in the pH of the alimentary canal, due to the production of lactic acid, which is one of the final products of the fermentation process carried out by these bacteria. This created an acidic environment that was unsuitable for the growth of aerobic and coliform bacteria, such as E. coli, which are important indicators of pathological intestinal bacteria (Mandalawi, 2005). We conclude from our research that beneficial changes in gut bacteria lead to improved health and performance in birds.

Conclusions

We conclude from our study that the water extract of clove flower powder led to a significant increase ($P \leq 0.05$) in the logarithmic numbers of Lactobacilli bacteria, the high levels of water extract of clove flowers powder (T4) gave the best results compared to the rest of the treatments.

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Authors contribution

Z.K.F. AL-Mhsenawi& **M.H.A. Al-Asadi**& **S. K.M. AL- hummod**: Collected the data and wrote the paper.

A.M. Al-Aboudi: Wrote the paper and performed the analysis.

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