

The Effect of Anise and Rosemary on the Microbial Balance in Gastro Intestinal Tract for Broiler Chicks

Ghalib Alwan Mohammed Al-Kassie, Mayada Fadil Mohammed,
Mohanad Falhi Hamood, Yasser Jamal Jameel

Department of Veterinary Public Health, Veterinary Medicine College, Baghdad University, Iraq

Abstract: This study was conducted to determine the effect of the addition of different percent levels of anise and rosemary, added to a standard diet, on the gastro intestinal tract. Total bacteria count, Coliform bacteria, lactobacilli bacteria and fungal count were determined in different parts of crop, jujinum and large intestine. Two hundred fifty day-old broilers (Arbor-Acre) were divided into groups of 50 birds each and randomly assigned to the five treatment groups. Each treatment has two replicates. Experiment were as follow: A control group with no anise and rosemary, and other four groups with the following additives, 0.5% anise, 1% anise, 0.5% rosemary and 1% rosemary. The data showed that the two additives (0.5% anise and 1% rosemary) had statistical effect ($P < 0.05$) regarding the decrease in the total bacteria count and Coliform count in crop, jujinum and large intestine compared with the control. In conclusion 0.5% anise and 1% rosemary used as antimicrobial balance in gastro intestinal tract for broiler chicks.

Key words: Anise, rosemary, antimicrobial, broiler

Introduction

Antibiotics have played a fundamental role in poultry production as growth and health promoters. One of these alternatives is related to dietary of poultry and that these use of aromatic plants to play as digestive enhancers (Williams and Losa, 2001). For this reason it's wise to look for exits subsequent antibiotics-resistant bacteria (Lee *et al.*, 2004).

Since ancient time, herbs have been known for their varying degrees of antimicrobial activity (Juven *et al.*, 1994; Change, 1995). More recently medical plants are used in food as natural antimicrobial (Del Campo *et al.*, 2000; Hsieh, 2000; Hsieh *et al.*, 2001).

The effects of herb medical on the performance of poultry production may be due to their characteristic flavors (Moleyan and Naeasimham (1992). Aromatic plants have been used traditionally in therapy of some diseases for a long time. Aromatic plants, anise (*Pimpinella anisum L.*), rosemary (*rosemarinus officia*) have been used as stimulating effects of digestion and antiparasitic, antibacterial on *Escherichia coli 0157* and *Salmonella typhimurium* (Cabuk *et al.*, 2003; Singh *et al.*, 2002; Tabanacal *et al.*, 2003) and antifungal (Soliman and Badea 2002).

Bowles and Miller, (1993) showed that aromatics are responsible for antibacterial activity, while the Farag *et al.* (1989 a,b) found these plants caused impairment of bacterial enzyme system. It was hypothesized that the characteristic odor and antibacterial action from feed intake .

The aim of this study is to use anise and rosemary on different percent levels in poultry nutrition as natural growth promoting substance instead of antibiotics and

to investigate its effect on intestinal microbial balance in gastro intestinal tract of digestive system.

Materials and Methods

Two hundred fifty one day-old broilers (Arbor Acre) were divided into five treatment groups of 50 birds each and randomly assigned to five treatment diets.

The experiment was carried out 42 days. Each treatment group was further sub-divided into two replicates of 25 birds per replicate. The presence and total count of bacterial, Coliform bacteria count, *lactobacilli* bacteria count and fungal count were the main factors tested in the gastro intestinal tract.

In the control group the birds were fed a standard diet (21.49% CP and 3188 kcal ME/kg). Two different percent levels of (0.5% and 1% anise) and (0.5% and 1% rosemary) added to the standard diets to generate the other four treatment groups. The diets were iso-caloric and isonitrogenous. The ingredient and chemical composition of the diets are presented in Table 1, were analyzed using AOAC (1990) procedure. The diet and water were provided *ad libitum*.

A photo period of 24 h/d in 4 weeks and 16 h/d in 4-6 week were maintained.

At the end of the experiment the birds are 6 weeks old. They were slaughtered by cutting the throat and the jugular vein with a sharp knife near the first vertebra from each of group (10 birds / group), were picked for eviscerating under sterilize condition. Samples were taken from the middle of the small intestine at the connection region of yolk sac with the small intestine (Rodimentary yolk sac stalks). A second sample was taken from crop and a third sample was taken from the

Al-Kassie *et al.*: Effect of Anise and Rosemary

Table 1: Composition of standard diet %

Feeds ingredients	1-42 day
Yellow corn	30.00
Wheat	32.50
Soybean meal (48% protein) ¹	19.50
Protein concentrate (50% protein)	10.00
Limestone	0.70
Salt	0.30
Oil	6.70
Vitamins and trace mineral premix ²	0.10
Methionine	0.10
Lysine	0.10
Total	100.00
Analysis	
Crude protein	21.49
Methionine + Cystine	0.33
Lysine	1.54
ME, cal kg ⁻¹	3188.00
Calorie protein ratio	1: 148.00

¹Source: Provime Company, Jordin, Premix:(1%) provided the following (per kilogram of complete diets) 1400 IU Vitamin A, 3000 IU Vitamin D3, 50 mg Vitamin E, 4 mg Vitamin K, 3 mg Vitamin B6, 6 mg Vitamin B12, 60 mg niacin, 20 mg pantothenic acid, 0.20 mg folic acid, 150 mg choline, 4.8 mg Ca, 3.18 mg P, 100 mg Mn, 50 mg Fe, 80 mg Zn, 10 mg Cu, 0.25 mg Co, 1.5 mg 10 dine

large intestine, then transit one ml from each samples were taken by sterilize glass bottles to the laboratory within ½ hours for determining the bacteria count (Total bacteria, Coliform bacteria, lactobacilli bacteria and fungal) according to Harrigan *et al.* (1976).

Data were analyzed using the General Linear Model procedure of SAS, (2001).

Duncan's multiple ranges test was used to detect the differences (P < 0.05) among different treatment means (Steel and Torrie, 1980).

Results and Discussion

Table 2 indicates the effect of the addition of different percent levels of anise and rosemary on the intestinal microbial balance of broiler chicks, that includes different parts of gastro intestinal tract of digestive system (crop, jujinum and large intestine) at six week old chicks.

The data showed that the two additives (0.5% anise and 1% rosemary) treatments, i.e. the second and fifth treatment respectively, have statistical effects (P < 0.05)

on the decrease of the total bacterial count and Coliform count in crop, jujinum and large intestine. It is worth noting that there are effects for the 1% anise and 5% rosemary treatments, i.e. third and fourth treatments, on the total bacterial count and Coliform count in crop, jujinum and large intestine. However all treatments should statistical effect (P < 0.05) in various compared with the control treatment one.

This may be considered as an indication to the fact that the addition of the 0.5% anise and 1% rosemary led to a significant decrease in the total aerobic bacteria count. These results are in agreement with that of Bolukbasi and Erhan, (2007), where they pointed out the positive effect in decreasing *E.coli* in various of gastro intestinal tract in order to improve the animal health.

In the control treatment, the Coliform bacteria count in the large intestine showed significantly statistical differences (P < 0.05).

The second and fifth treatments had statistical significant differences (P 0.05) compared with the third and fourth treatments.

The second treatment 0.5% anise gave minimum value compared to total of the remaining treatments. For the jujinum region the bacterial count for Coliform bacteria (control) exceeded significantly (P < 0.05) with respect to the second and fifth treatment. On the other hand there was no significant differences between the first, third and fourth treatment.

In general Coliform bacteria existed in the content of the large intestine naturally and the crop in the beginning of the bird age. The effects of the active ingredient that exist in anise and rosemary (anethol and borned) into digestive system were reported (Kim *et al.*, 2004 ; Cabuk *et al.*, 2003) and antipyretic effects were also reported (Feng and Lipton, 1987; Afifi *et al.*, 1994).

The Coliform bacteria are considered one of the health indicator in the status of the gastro intestine tract then presence in high contents in the treatments of anise and rosemary are indicators of the importance of the active material on inhibiting the bacteria growth. These materials act in a way or other on the variation in the building of cellular wall of the bacteria which lead to the

Table 2: The addition of different percent levels of anise and rosemary on the intestinal microbial balance of broiler chicks (CFU gm⁻¹)

Characteristic studies Treatments	Large Intestine				Small Intestine (Jujinum)				Crop			
	Fungal count	<i>Lactobacilli</i> count	Coliform count	Total count	Fungal count	<i>Lactobacilli</i> count	Coliform count	Total count	Fungal count	<i>Lactobacilli</i> count	Coliform count	Total count
T1 control	2.4±	1.40±	5.70±	8.63±	3.63±	1.24±	5.45±	9.62±	2.10±	0.64±	7.68±	9.67±
T2 anise 0.5%	00.04 ^a	0.05 ^a	0.06 ^a	0.03 ^a	0.17 ^a	0.04 ^b	0.06 ^a	0.14 ^a	0.04 ^a	0.02 ^a	0.09 ^a	0.04 ^a
T3 anise 1%	2.30±	1.38±	5.30±	8.50±	3.60±	1.29±	5.18±	9.25±	2.17±	0.73±	7.37±	9.53±
T4 rosemary 0.5%	0.09 ^a	0.04 ^a	0.15 ^a	0.05 ^b	0.23 ^a	0.03 ^{ab}	0.16 ^a	0.04 ^{bc}	0.06 ^a	0.05 ^a	0.08 ^a	0.04 ^b
T5 rosemary 1%	2.32±	1.35±	5.67±	8.51±	3.73±	1.28±	5.32±	9.55±	1.95±	0.65±	7.63±	9.58±
T1 control	0.05 ^a	0.07 ^a	0.06 ^{ab}	0.06 ^{ab}	0.15 ^a	0.05 ^a	0.06 ^{ab}	0.05 ^{ab}	0.11 ^{ab}	0.08 ^a	0.09 ^{ab}	0.02 ^{ab}
T2 anise 0.5%	2.37±	1.47±	5.45±	8.50±	3.37±	1.38±	5.28±	9.58±	1.68±	0.73±	7.47±	9.65±
T3 anise 1%	0.07 ^a	0.04 ^a	0.09 ^{bc}	0.04 ^b	0.05 ^a	0.05 ^{ab}	0.05 ^{ab}	0.04 ^{ab}	0.05 ^a	0.03 ^a	0.06 ^{bc}	0.05 ^{ab}
T4 rosemary 0.5%	2.45±	1.42±	5.38±	8.37±	3.48±	1.43±	5.17±	9.20±	1.85±	0.75±	7.20±	9.52±
T5 rosemary 1%	0.08 ^a	0.03 ^a	0.08 ^c	0.03 ^c	0.09 ^a	0.09 ^a	0.13 ^b	0.04 ^c	0.13 ^{bc}	0.03 ^a	0.08 ^c	0.05 ^b

creation of gaps inside the bacteria cytoplasm to the outside region.

This in turn has negative effect on the vitality of that bacteria (Lee *et al.*, 2004).

Bolukbas and Erhan (2007) started the mechanism of the bacterial inhibition effect of aromatic plant through the resulting interference between the contents of anise and rosemary with cellular membrane for microbial germ which led to a change in the diffusion of ion K, H via viability of micro-organism.

To the best of my knowledge this is the first time the effect on microbial balance in gastro intestine of poultry is investigated.

References

- Afifi, N.A., A. Ramadan, E.A. El-Kashoury and H.A. El-Banna, 1994. Some pharmacological activities of essential oils of certain umbelliferous fruits. *Vet. Med. J. Giza*, 42: 85-92.
- A.O.A.C., 1990. Official Methods of Analysis Association of Agricultural Chemists, Virginia, D.C., USA, 746-780.
- Bowles, B.L. and A.J. Miller, 1993. Antibotulinal properties of selected aromatic and aliphatic aldehydes. *J. Food Prot.*, 56: 788-794.
- Bolukbasi, S. and M. Erhan, 2007. Effect of dietary thyme (*Thymus vulgaris*) on laying hens performance and *Escherichia coli* (*E. coli*) concentration in feces. Ataturk University, Faculty of Agriculture, Department of Animal Science, 25240, Erzurum, Turkey.
- Cabuk, M., Alcicek, M. Bozkurt and N. Imre, 2003. Antimicrobial properties of the essential oils isolated from aromatic plants and using possibility as alternative feed additives II. National Animal Nutrition Congress, 18-20, September, PP: 184-187.
- Del Campo, J., M.J. Amiot and C. Nguyen, 2000. Antimicrobial effect of rosemary extract. *J. Food Protec.*, 63: 1359-1368.
- Farag, R.S., Z.Y. Daw, F.M. Hewed and G.S.A. El-Baroty, 1989a. Antimicrobial activity of some Egyptian Spice essential oils. *J. Food Protec.*, 52: 665-667.
- Farag, R.S., Z.Y. Daw and S.H. Abo-Raya, 1989b. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *J. Food Sci.*, 54: 74-76.
- Feng, J. and J.M. Lipton, 1987. Eugenol:Antipyretic activity in rabbits. *Neuropharmacology*, 26: 1775-1778.
- Harrigan, W.F. and M.E. McMane, 1976. *Laboratory Methods in Microbiology*. Academic press. London, UK.
- Hsieh, P.C., 2000. Antimicrobial effect of cinnamon extract. *Taiwanese J. Agric. Chem. Food Sci*, 38: 184-193.
- Hsieh, P.C., J.L. Mau and S.H., 2001. Antimicrobial effect of various combinations of plant extract. *Food Microbial.*, 18: 35-43.
- Juven, B.J., J. Kanner, F. Schved and H. Weisslow, 1994. Factors that interact with antibacterial action of thyme essential oil and its active constituents. *J. Appl. Bacteriol.*, 76: 626-631.
- Lee, K.W., H. Everts, H.J. Kappert and A.C. Beynen, 2004. Growth performance of broiler chickens fed a carboxy methyl cellulose containing diet with supplemental carvacrol and/or cinnamaldehyde. Dep. Of Nut., Fac. of Vet. Med. Utrecht University, 3508. TD. Utrecht, Netherlands.
- Moleyan, V. and P. Narasimham, 1992. Antibacterial activity of essential oil components. *Int. J. Food Microbial.*, 16: 337-342.
- SAS, 2001. *SAS / STAT Users Guide for Personal Computer*. Release 6.18. SAS Institute, Inc., Cary, N.C., USA.
- Singh, G.I.P. Kappoor, S.K. Pandey, U.K. Singh and R.K. Singh, 2002. Studies on essential oils: Part 10; antibacterial activity of volatile oils of some species *phytother Res.*, 16: 680-682.
- Soliman, K.M. and R.I. Badea, 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food chem. Toxicol.*, 40: 1669-1675.
- Steel, R.G.D. and J.H. Torrie, 1980. *Principle and Procedures of Statistics*. 2th ed. McGraw-Hill book Co. Inc. New York.
- Tabanca, N.E. Bedir, N. Kirmer, K.H. Baser, S.I. Khan, M.R. Jacob and I.A. Khan, 2003. Antimicrobial compounds from *Pimpinella* species growing in Turkey. *Planta Med.*, 69: 933-938.
- Williams, P. and R. Losa, 2001. The use of essential oils and their compounds in poultry nutrition. *World Poult.*, 17: 14-15.