The effect of Mentha extract(*Mentha piperita*) on immune response in Broiler Chickens

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Abstract: This experiment was carried out to determine the effects of Mentha extract *(Mentha piperita)* on the relative weight of the immune organs and serum antibody titer against Newcastl disease viruse and infectious Bronchitis viruse. One hundred and sixty mixed one-day-old broiler chicks (Ross 308) in four groups and 10 birds for each based on a completely randomized design were used. All the chickens were fed the similar starter (day 1-21 of age) and grower (day 22-42 of age) diets in pellet form, but received 0.0 (ZM), 0.2 (LM), 0.4 (MM) and 0.6% (HM) alcoholic extract of Mentha piperita in drinking water during the experimental period. At 21 and 42 days of age, two birds per pen were selected, weighed and killed by decapitation to obtain the immune organs relative weight such as spleen and bursa fabricius. Consequently blood samples were collected and blood serum was separated to measure of Newcastle and Bronchitis disease viruses antibody titers by using the elaisa reader. All experimental data were subjected to the GLM procedure of SAS as a complete randomized design and the mean values were compared by Tukey Multiple Range Test ($P<0.05$). No significant difference between treatments was observed for the measured factors at 21 and 42 days of age. The results of this study revealed that Mentha extract consumption in drinking water could not stimulate the immune system response in broiler chickens.
[Rahim Abdulkarimi and Farzad Abdullahzadeh .,The effect of Mentha extract(*Mentha piperita*) on immune response in Broiler Chickens. Journal of American Science 2011;7(12):82-85]. (ISSN: 1545-1003). http://www.americanscience.org. 12

Key words: Mentha piperita, immune response and broiler chickens.

1. Introduction

Antimicrobial resistance in the internal bacteria of animal including *Salmonella*, *Escherichia coli* (*E.coli*), *Enterococci*,etc, caused by the antibiotic growth promoters consumption is of special concern to human health because is likely to transfer from the food chain to humans (Endtz et al., 1991) and consequently usage of this components is restricted or baned. The phasing out of antibiotic growth promoters (AGP) will affect the poultry and animal industry at large. To minimize the loss in growth, there is a need to find alternatives to AGP (Thakare, 2004). There are a number of non-therapeutic alternatives such as enzymes, inorganic acids, probiotics, prebiotics, herbs, immunostimulant and other management practices (Banerjee, 1998). Medical plants and their principal secondary metabolits, used extensively in food products, perfumery, and dental and oral products due to their different medicinal properties (Suppakul et al.,2003), are the most common materials that are applied instead of Antibiotic growth promoters in poultry production(Greathead, 2003).

As regarded in current years, epidemic infectious diseases are important problam in throughout world and the cuase of the financial failure for the poultry producers. In addition, other factors such as vaccination failure, infection by immune suppressive diseases, and abuse of antibiotics can induce immunodeficiency. Utilization of immunostimulants is one solution to improve the immunity of animals and to decrease their susceptibility to infectious disease (Liu, 1999). In some studies, medical plants efficiency on broiler immune system has been reported. Dietary birds with polysavone (alfalfa extract) improved the relative thymus, bursa and spleen weights and led to increase in proliferation of T and B lymphocytes compared with the control group ($P<0.05$). Moreover polysavone consumption resulted in a significant increase ($P<0.05$) in serum antibody titer of Newcastle disease virus (Dong et al., 2007). Khaligh et al (2011) indicated that addition of 10 g/kg a blend of alfalfa, liquorice root, great burdock, cinnamon to the broiler diet resulted in the most consistent improvement in antibody titer against Newcastle disease virus ($p < 0.05$) compared with the control.

Mentha piperita is one of the world's oldest medicinal herbs and used in both Eastern and Western traditions. This plant is a perennial plant in *Lamiaceae* family and contains about 1.2-1.5% *essential oils*. The principal components of the oil are menthol (35-55%), menthone (20-30%) and menthyl acetate (3-10%) (Escop, 2003).

Some papers have reported the beneficial effects of menthe pipperita on performance in broiler

chickens (Galib and Al-Kassie, 2010; Hosseini-Mansoub, 2011) But evidence about the effect of mentha extract on immune response in broiler chickes is not sufficient and therefore the aim of this study was to evaluate the effect of mentha piperita extract on immune system response in drinking water.

2. Materials and Methods

A total of 160 day-old mixed sex broiler chicks (Ross 308) were weighed and based on completely randomized design assigned to 4 treatment groups with 4 replicate and 10 bird (5 male and 5 female) per each. Water and feed were provided *ad libitum* for consumption. All the chickens were fed the similar starter (day 1-21 of age) and grower (day 22-42 of age) diets in pellet form (Table 1), but the drinking water of the birds supplemented with 0.0 (ZM), 0.2 (LM), 0.4 (MM) and 0.6% (HM) alcoholic extract of Mentha piperita during the whole of experimental period.

Mentha piperita alcoholic extract was prepared using a standard maceration method (Zhang et al., 2005). For this purpose, vegetative parts of the shade dried menthe piperita full bloom stage were crushed and soaked in ethanol 80% in 1:5 ratios (w/v) for 72 h on a shaker. All treatments (drinking water) were prepared daily. Bronchitis vaccination against bronchitis virus was done on the 1th and 14 th days (as eye drop), and vaccination against Newcastle virus happened by injection in breast muscle on 8 th day of the experimental period. At 21 and 42 days of age, two birds per pen (a male and a female) were selected, weighed and killed by decapitation to obtain the immune organs relative weights such as spleen and bursa Fabricius (percentage of live body weight). Blood samples were collected in anticoagulant tubes (citrate sodium 3.6% solution) during a forty minute period. After centrifugation (5000 rpm) for 7 min, Blood serum was separated and then Newcastle and bronchitis disease virus antibody titers were measured by using the elaisa reader device (Ornest American staff, fax 3200).

The data were subjected to the GLM procedure of SAS statistical software (version 9.1) and analyzed based on a completely randomized design. When the overall model was statistically different ($P<0.05$), the Tukey-Kramer multiple comparison test was used to compare the mean values ($P<0.05$). Moreover, orthogonal contrasts were constructed in order to compare the mean response variables for mentha extract received birds vesus the control birds.

3. Results & Discussion

The effect of dietary mentha extract supplementation in drinking water on immune system of broiler chickens is summarized in table 2. No significant differences between treatments was found for the measured factors at 21 and 42 days of age. The results of this study revealed that Mentha extract consumption in drinking water could not stimulate the immune system response in broiler chickens. In agreement with our results, a herbal extract with a blend of cinnamon, thyme and oregano didn't has a significant effect on the serum concentration of immunoglobulin G in pigs (Namkung et al. 2004). In same way, serum antibody titer level against NDV in broilers that supplemented with 0.2, 0.4, 0.6, 0.8 and 1% garlic powder didn't differe with control birds at 14, 28 and 42 days of age (Pourali et al., 2010).

Table 1. Composition of experimental diets

Ingredients (%)	Starter (0-21 d)	Grower (21-42 d)
Corn	54.87	61.78
Soybean meal (44 % protein)	36.72	26.36
Fish meal	1.31	4.50
Vegtable oil	3.00	4.00
Limestone	1.15	1.05
Dicalcium phosphate	1.94	1.49
Vit. and min. premix[1]	0.50	0.50
Salt	0.30	0.30
DL-methionine	0.21	0.02
Total	100.00	100.00
Calculated analysis		
ME (kcal/kg)	2937	3100
CP (%)	21.44	19.37
Calcium (%)	1.05	1.00
A. Phosphorus (%)	0.51	0.50
Sodium (%)	0.16	0.14
Arginine (%)	1.41	1.23
Methionine + Cystine (%)	0.91	0.69
Lysine (%)	1.20	1.10
Tryptophan (%)	0.31	0.26

[1] provide per kilogram of diet: vitamin A, 15000 IU; vitamin D_3,8000 IU; vitamin K3, 3 mg; B_{12}, 15 µg; niacin, 32 mg; choline, 840 mg; biotin, 40 µg; thiamine, 4 mg; B_2 (riboflavin), 6.6 mg; pyridoxine, 5 mg; folic Acid, 1 mg; Zn, 80 mg; Mn, 100 mg; Se, 200 mg; Fe, 80 mg; Mg (magnesium oxide), 12; Cu, 10 mg; Ca (calcium pontatenate), 15 mg; iodeine,1 m

In the some studies that carryed out in animal fields, the influnce of medical plants on improvement of immune system had been reported (Rivera et al. 2003; Song et al. 1998; Djeraba and Quere 2000; Sharma et al. 1996; Savoini et al. 2003; Schuberth et al. 2002; Grossi et al. 2004). Furthermore, Rahimi et al (2011) reported that dietary thyme extract (0.1%) soluble in water did not affect immune system, but relative weight of bursa

fabricius significantly affected by garlic group and antibody response to Sheep Red Blood Cells (SRBC) was higher in coneflower group compared with control group ($P<0.05$). Nickels (1996) mentioned that peppermint oil maintains the structural integrity of immune cells due to its strong antioxidant action which protects cell membrane from free radical oxidants, thereby resulting in an improved immune response. Barbour and Danker (2005) reported that essential oils of eucalyptus and peppermint improved the homogeneity of immune responses and performance in MG/H9N2-infected broilers.

Awaad et al (2010) indicated that eucalyptus and peppermint oils are able to improve both innate-cell mediated and humoral immune response in chickens. According to Mekay and Blumberg (2006), peppermint oil has a significant antimicrobial, antitumor, antiviral, immunomodulating and chemopreventive potential. Also Iscan *et al*. (2002) and Schuhmacher *et al*. (2003) reported that peppermint oil had antimicrobial effects against wide range of bacteria which improves the general healthy conditions of animal that may be reflected in increased immune response.

Considering the menthe piperita characteristics, we anticipated that an increase in immune response of chicks would be observed. The low results of menthe extract on immune system is probably related to the dose of additives, type of mentha, posses and preparation period and also vaccination program times and stimulator material that used in our study. Regarding this fact that a few reports are available on the impact of mentha or mentha component such as mentol and menton on bird immune response, more studies will be needed to investigate mentha extract immonomodulatory properties and principal components on broiler health. In conclusion, results of the present study showed that supplementation of 0.2, 0.4 and 0.6% mentha extract in drinking water did not improve the immune status in broiler chickens in the whole experimental period.

Table 2. Effects of different levels of mentha extract supplemented in drinking water on the immune organs and serum antibady titer against Newcastl disease viruse and infectious Bronchites viruse of broiler chickens at 21 and 42 days of age

Parameter	Antibady titer against NDV		Antibady titer against IBV		Bursa fabricius (%)		Spleen (%)	
Treatmens	21 d	42 d	21 d	42d	21d	42d	21d	42d
Control (0.0)	514	884	490	578	0.54	0.1	0.09	0.11
LM(0.2%)	451	2128	505	506	0.55	0.09	0.11	0.1
MM(0.4%)	507	1865	655	671	0.54	0.09	0.1	0.1
HM(0.6%)	1161	1525	518	506	0.54	0.11	0.09	0.11
P value	11.0	290.	0.58	0.25	0.99	0.59	0.13	0.85
[1]*Orthogonal comparisions*								
A versus B	0.47	0.09	0.53	0.32	0.98	0.84	0.6	0.99

1- Mentha extract received birds (A), Control group (B)

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11/11/2011