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## Use of Habek Mint (*Mentha longifolia*) in Broiler Chicken Diets

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**Abstract:** An attempt has been conducted to evaluate the effect of habek on performance and immunity of broiler chickens. Five levels of whole habek, 0, 25, 100, 150 and 200 g/kg were incorporated into basal diet of 125 broilers for 5 weeks. The results of the study showed that including 150 g/kg habek into broiler diet make a significant improvement in the mean body weight, daily average gain, feed intake and food conversion ratio. However, in another experiment, habek had no effect on the immune response of the birds against Newcastle disease virus live vaccine when a group of 25 birds was given basal diet supplemented with 150 g/kg habek compare to that fed basal diet only.

**Key word:** Habek mint, *Mentha longifolia*, growth promoters, broilers, Saudi Arabia

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### Introduction

*Mentha* species - of the family *Jabiateae* - are well known in traditional medicine (Lewis and Elvin-Lewis, 1977). Habek mint (*Mentha longifolia*) – known as horse or wild mint - like many other members of this genus, is often used in domestic herbal remedy, being valued especially for its antiseptic properties and its beneficial effects on the digestion (Foster and Duke, 1999). The active virtues of the habek depend on the abundant volatile oil, which has been found to contain a hydrocarbon, thymol, and higher oxygenated compounds. It yields its virtues to boiling water, but particularly to alcohol. The main medicinal action of the leaves and flowering stems are antispasmodic, choleric, carminative and CNS stimulant (Grieve, 1981; Chopra *et al.*, 1992). The leaves contain about 0.75% essential oils (Eos) (Chopra *et al.*, 1992), which is sometimes used as a substitute for peppermint oil in confectionery (Bown, 1995). The composition of Eos obtained from habek exhibit strong antibacterial (Kaushik *et al.*, 2003; Mimica-Dukic *et al.*, 2003) and antioxidant activities (Mimica-Dukic *et al.*, 1999). The presented paper was carried out to investigate the effect of incorporating habek in basal diet of broilers on the overall performance, and immunity of the birds.

### Materials and Methods

**Experimental birds:** A total of 225 Cobb broiler chicks were used in this study. One-day-old chicks were obtained from a local hatchery (Al-Ahsa, Saudi Arabia) and placed in a closed house; at the Agricultural and Veterinary Training and Research Station of King Faisal University in Al-Ahsa. Strict sanitation practices were employed to the house before and during the course of the experiment. The house was divided into five small pens each measuring 4.0 m<sup>2</sup>. Each pen was provided

with separate feeding and watering facilities. Saw dust used as litter and artificial lighting provided 24 hours a day. The temperature of the house and vaccination program applied based on broiler raisers' recommendations.

**Experimental diet:** The basal diet (Table 1) was formulated to meet nutrient requirements of broiler chickens (NRC, 1996). Fresh habek was obtained from local market, dried and added in a powdered form to the basal diet according to the experimental plan.

**Plantation:** Habek is cosmopolitan herb. It is indigenous to Eastern Europe, Middle East, South and North Africa and grows wild in southern Saudi Arabia, but domesticated in gardens and fields. The herb is perennial that has creep long an under ground root stock. It is fast and easy growing plant; especially in most soil and situation as long as the soil is not too dry. It grows well in heavy clay soils with fairly aggressive spreading roots. Division can be easily carried out at almost any time of the year, though it is probably best done in the spring or fall to allow the plant to establish more quickly. They will quickly establish and become planted out in the summer. The plant grows between 0.5-1 m high and may reach up to 1.5 m in favorable condition. The soft, lanceolate leaves are between 45-100 mm long and 7-20 mm wide, varies from light and dark green to grey in color and formed in pairs opposite each other along the square-shaped stem. The leaves are harvested as the plant comes into flower and can be dried for later use. They are strong and aromatic odor, with pungent and slightly bitter taste.

**Experimental design:** Two subsequent trials were carried out in this study:

Table 1: Composition of the basal diet<sup>1</sup>

Ingredients and composition	Amount (g/kg)
Yellow corn	567.0
Soybean meal (44% protein)	352.0
Corn oil	35.0
Limestone	12.0
Sodium chloride	3.8
Calcium phosphate, dibasic	21.0
Vitamins & trace mineral premix <sup>2</sup>	2.2
Methionine	2.2
Lysine	4.0
Anti Oxidant	0.8
Composition	
Dry matter (%)	84.92
Ash (%)	2.96
Crude protein (%)	21.03
Crude fat (%)	5.64
Crude fiber (%)	4.11
Metabolize energy (Kcal/kg)	3005.00
Calcium (%)	1.01
Available phosphorous (%)	0.75
Niacin (mg/kg)	63.24
Carotene (mg/kg)	1.13
D-Pantothenate (mg/kg)	13.91
Methionine (%)	0.54
Cystine (%)	0.31
Tryptophane (%)	0.30
Isoleucine (%)	1.09
Leucine (%)	1.76
Thyronine (%)	0.79
Glycine (%)	1.03
Phenylalanine (%)	1.02
Valine (%)	1.08
Histidine (%)	0.49
Lysine (%)	1.51
Arginine (%)	1.49

<sup>1</sup>Source: ARASCO, Riyadh, Saudi Arabia

<sup>2</sup>Premix (1%) provided the following (per kilogram of complete diet); 13,200 IU vitamin A (retinal acetate), 3,750 IU vitamin D<sub>3</sub>, 66 IU vitamin E (*DL-α-tocopheryl* acetate), 2 mg vitamin K<sub>3</sub> (menadione bisulphate), 4 mg vitamin B<sub>1</sub>, 13.2 mg vitamin B<sub>2</sub>, 7.9 mg vitamin B<sub>6</sub>, 12 µg vitamin B<sub>12</sub>, 0.253 mg biotin, 2.2 mg folic Acid, 120 mg manganese from MnSO<sub>4</sub> · H<sub>2</sub>O, 80 mg iron from FeSO<sub>4</sub> · H<sub>2</sub>O, 10 mg, copper from CuSO<sub>4</sub> · 7H<sub>2</sub>O, 120 mg, zinc from ZnSO<sub>4</sub>, 2.5 mg iodine from CaIO<sub>4</sub>, 1 mg cobalt from CoSO<sub>4</sub>, 0.2 mg selenium diet as Na<sub>2</sub>SeO<sub>3</sub>, 100 mg ethoxyquin.

**Experiment 1:** On arrival at the site of the experiment, 125 chicks were allocated at random into five groups of 25 chicks each. Birds in group 1 were only fed the basal diet and assigned as untreated control. Birds in groups 2, 3, 4, and 5 were fed the basal diet that was supplemented with habek at 25, 100, 150 and 200 g/kg, respectively. The chicks of each treatment were fed the respective diets and water was provided *ad libitum* throughout the experimental period.

Birds were monitored for clinical signs and mortality and recorded in a daily basis. Mean body weights (MBW) and food consumption were measured weekly throughout the experimental period. Average daily weight gains (ADG), daily feed intake (DFI) and food conversion ratio (FCR) were calculated and recorded. Toward the end of the fifth week, the experiment was terminated and the birds were slaughtered and processed for human consumption. Three samples from each group were randomly selected for analysis of breast and thigh muscles contents according to methodology described by AOAC (1984). Twenty other birds from each group were coded and blindly distributed for assessment by a panel of 20. A questionnaire was designed and used for collection data.

**Experiment 2:** In this experiment the only amount of habek (150g/kg) that gave overall best performance in experiment 1 was used in this experiment. The aim was to evaluate the effect of habek feed additive on the response of immune system of broilers to Newcastle disease virus (NDV) live vaccine. A total of 100 chicks were randomly divided into 4 equal groups, Groups A and B was fed basal diet alone and groups C and D were fed basal diet supplemented with 150g/kg habek. Management and sanitation practices used in Experiment 1 had been implemented in this part. At day 18 of chicks' age, groups B and D were vaccinated with live vaccine of NDV by eye drop. Groups A and C unvaccinated as shown in the following scheme:

Group	Treatment with	Vaccination
N=25	150 g/kg habek	with NDV
A	No	No
B	No	Yes
C	Yes	No
D	Yes	Yes

**Blood collection and serum separation:** Blood was collected from each bird via brachial vein at days; 14, 21, 28 and 35 of age. Sera were separated, labeled and stored at -20°C until further analysis.

**ELISA procedure:** Batches of sera were subjected to serological test. Antibody titers against NDV were measured using ELISA technique described by Snyder *et al.* (1984) and NDV antibody test kit (Synbiotics Corporation, San Diego, USA).

**Statistical analysis:** Analysis of variance using general linear model (GLM) procedure in the PC-SAS<sup>®</sup> (1988) was used to estimate the variations among the means. Comparison of means in different groups was made by Duncan's multiple-range test (Steel and Torrie, 1980). P<0.05 was accepted as statistically significant.

## Results

**Performance of the birds:** Differences in MBW (g), ADG

Table 2: Effect of different levels of habek, incorporated, in a basal diet on body weight, weight gain, feed intake and feed conversion ratio of group of 25 broilers

Parameter	Group				
	1	2	3	4	5
Initial body weight (g)	36.00	36.00	36.00	37.00	34.00
Body weight (g)					
week 1	115.63	117.40	118.99	115.83	112.62
week 2	281.01	282.43	287.16	291.88	278.19
week 3	508.50 <sup>ab</sup>	509.48 <sup>ab</sup>	507.80 <sup>ab</sup>	521.20 <sup>a</sup>	483.50 <sup>b</sup>
week 4	879.68	888.70 <sup>b</sup>	934.08	963.70 <sup>a</sup>	865.68
week 5	1290.10	1364.39 <sup>b</sup>	1361.90 <sup>b</sup>	1489.05 <sup>a</sup>	1316.40
Average daily gain (g/d)					
week 1	11.38	11.62	11.86	11.26	11.23
week 2	17.50	17.60	17.94	18.21	17.44
week 3	22.50 <sup>ab</sup>	22.55 <sup>ab</sup>	22.47 <sup>ab</sup>	23.06 <sup>a</sup>	21.40 <sup>b</sup>
week 4	30.13 <sup>b</sup>	30.45 <sup>b</sup>	32.07 <sup>ab</sup>	33.10 <sup>a</sup>	29.70 <sup>c</sup>
week 5	35.83 <sup>c</sup>	37.95 <sup>b</sup>	37.88 <sup>b</sup>	41.49 <sup>a</sup>	36.64 <sup>bc</sup>
Feed intake (g/bird/d)	85.14 <sup>bc</sup>	84.70 <sup>bc</sup>	86.78 <sup>b</sup>	82.69 <sup>c</sup>	89.49 <sup>a</sup>
Feed conversion ratio (g/g)	2.38 <sup>ab</sup>	2.23 <sup>bc</sup>	2.29 <sup>b</sup>	2.00 <sup>c</sup>	2.44 <sup>a</sup>

Means within a row with different superscripts differ significantly (P < 0.05)

Table 3: Crude protein (%) and ether extract (%) of broilers breast and thigh muscles fed basal diet after incorporated of different levels of habek

Content (%)	Group				
	1	2	3	4	5
Breast	0.63	0.59	0.62	0.61	0.59
Fat					
Thigh	2.49	2.53	2.38	2.47	2.44
Breast	22.21	22.20	21.90	21.90	22.00
Protein					
Thigh	19.49	19.13	18.94	19.22	18.89

(g/d), DFI (g/bird/d) and FCR (g/g) between the different groups of the experiment were illustrated in Table 2. A significant increase (P<0.05) in MBW and ADG of group 4 in weeks 3, 4 and 5 of age was concluded compared to the other groups at the same periodical designations. However, group 5 showed significant decrease (p<0.05) in MBW and ADG compared to the groups, 2, 3 and 4. DFI and FCR of group 4 of birds showed a significant reduction (P<0.05) compared to other groups. Mortality was negligible as it was not affected by treatment.

**Carcass analysis and organoleptic test:** Protein and fat contents in breast and thigh muscles were not influenced by habek (Table 3). Likewise, when organoleptic test was made, it revealed that habek did not induce any abnormal color, odor, or flavor (Table 4).

**Immune response to NDV live vaccine:** Mean antibody titer (MAT) between groups was shown in Fig. 1. MAT at week 2 of age were nearly the same of all groups. It increased significantly (P<0.05) in groups B and D at

weeks 4 and 5 of age compared to groups A and C. However, there were no significant differences between groups B and D, and between groups A and C (P>0.05) were concluded.

### Discussion

Increasing dietary habek from 0 to 150 g/kg increased the MBW and ADG by about 15 and 16%, respectively and improved DFI and FCR by about 3 and 19%, respectively (Table 2). The significant improvement in the overall performance of broilers fed habek supported an earlier hypothesis suggests that the herb is valued for its beneficial effect on the digestion (Grieve, 1981; Chopra *et al.*, 1992).

Chemical analyses revealed that the major components of Eos isolated from habek were; carvone, piperitenone oxide, piperitone oxide,  $\alpha$ -caryophyllene, germacrene, limonene and trans-piperitol (Nori-Shargh *et al.*, 2000; Monfared *et al.*, 2002; Rasooli and Rezaei, 2002). The analysis indicated variations in quantity and quality of the components among those collected from different geographical areas (Mimica-Dukic *et al.*, 1996; Venskutonis, 1996; Karousou, 1998; Baser *et al.*, 1999; Abu Al-Futuh *et al.*, 2000; Nori-Shargh *et al.* 2000; Ghoulami, 2001; Monfared *et al.*, 2002; Rasooli and Rezaei, 2002).

Pharmacological properties of habek Eos were significantly increased intestinal motility and total bile secretion and improved hepatic antioxidant status in mice by decreasing lipid peroxidation, increasing hepatic glutathione and superoxide dismutase activity (Larson, 1988; Mimica-Dukic *et al.*, 1993a, 1999).

Microbiologically, habek Eos had powerful antimicrobial and antifungal activities against certain microorganisms

Table 4: Organoleptic test for carcass of broilers fed on a diet supplemented with different levels of habek assessed by a panel of 20

Meat	Evaluation scheme		Group					
			1	2	3	4	5	
Raw	Color	Breast	L	0	0	0	0	0
			S	20	20	20	20	19
			D	0	0	0	0	1
		Thigh	L	0	0	2	0	1
			S	20	20	15	18	15
			D	0	0	3	2	4
Cooked	Color	Breast	L	0	0	0	0	0
			S	20	20	20	20	20
			D	0	0	0	0	0
		Thigh	L	0	0	0	0	0
			S	20	20	20	20	20
			D	0	0	0	0	0
	Odor		N	20	20	20	20	20
			U	0	0	0	0	0
	Flavor		A	15	14	13	14	14
			B	5	6	7	6	6
			C	0	0	0	0	0
	Tenderness		A	14	14	15	14	13
B			6	5	5	6	6	
C			0	1	0	0	1	

L (lighter), S (similar), D (darker); in color compared to normal commercially available broiler meat.

N (normal smell), U (Abnormal smell); Flavor and tenderness assessment: A= very good, B= good, C= fairly acceptable.

known to be pathogenic to broiler chickens, particularly, *E. coli*, *Clostridium perfringens*, *Staphylococcus aureus*, *Streptococcus spp.* and *Candida albicans* (Solaiman *et al.*, 1998; Sökmen *et al.*, 2000, Rasooli and Rezaei, 2002; Kaushik *et al.*, 2003; Mimica-Dukic *et al.*, 2003). Carvone, thymol and p-cymene (compounds identified in Eos of habek) showed strong antimicrobial activity (Mimica-Dukic *et al.*, 1993b; Kokkini *et al.*, 1995; Matovc and Lavadinovic, 1999). Antimicrobial drugs have often employed in poultry industry for therapeutic and prophylactic purposes in the case of growth promoters (Brander, 1985). Therefore habek antimicrobial substances may act as growth promoter, which in turn inhibit intestinal pathogenic organisms and improve digestion and absorption.

Thymol is able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane (Helander *et al.*, 1998). Such conclusion was achieved by Juven *et al.* (1994) who examined the working of thymol against *Salmonella typhimurium* and *Staphylococcus aureus* and hypothesized that thymol binds to membrane proteins hydrophobically, thereby changing the permeability characteristics of the membrane. Further study indicated that the specific growth rate of *E. coli* and *Streptococcus thermophilus*

decreased with increasing concentrations of carvone, which suggests that it acts by disturbing the metabolic energy status of cells (Oosterhaven *et al.*, 1995).

The philosophy of using crude habek herb in this study was based on conclusions of an earlier studies indicated that the whole Eos have a greater antibacterial activity than the major components mixed (Gill *et al.*, 2002; Mourney and Canillac, 2002), and suggests that the minor components are critical to the activity and may have a synergistic effect or potentiating influence. This synergism was demonstrated by Ultee *et al.* (2002; 2000) who found p-cymene is not an effective antibacterial when used alone, but when combined with carvacrol, synergism has been observed against *Bacillus cereus*.

The substantial improvement of broilers' overall performance after feeding basal diet incorporated with 150g/kg habek could be attributed to the antioxidant properties of the herb that may stimulate protein synthesis by bird enzymatic system, as well as to the effect of some antimicrobial components which may act as growth promoters. However, the depression in performance of birds fed 200 g/kg habek appears less likely to be attributed to the palatability, since FI (g/bird/d) and FCR (g/g) were significantly higher (P<0.05) in this group compared to the other groups (Table 2). Further

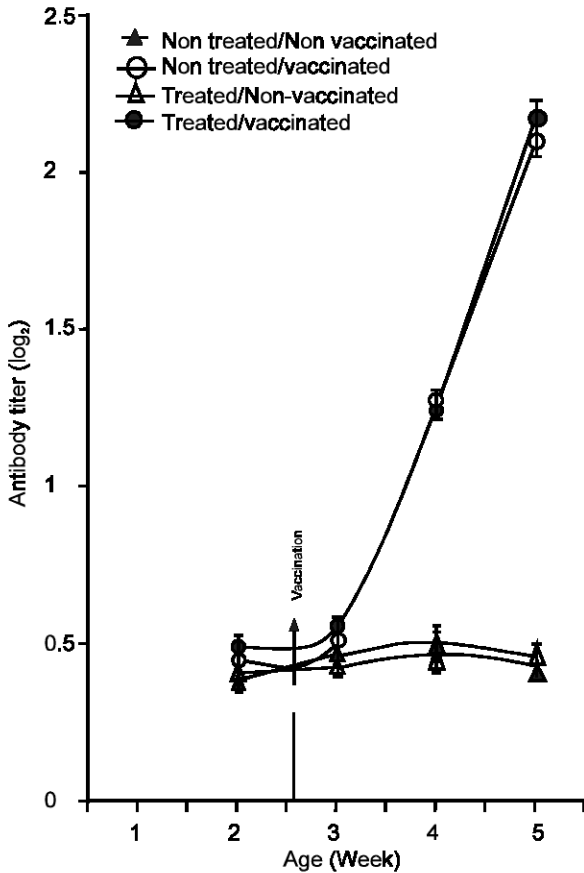


Fig. 1: Antibody titer (mean±SD) against NDV live vaccine for broilers fed either non-supplemented or supplemented basal diet 150g/kg habek

studies might be considered to elucidate the effect of different doses of habek on the metabolism.

With regard to MAT against NDV live vaccine, the serological responses showed no evidence that habek had stimulated or suppressed the immune system of the bird. For clear conclusion, further investigation is preferable to study the effect of the herbs on major immune system elements.

In general, better performance was seen when 150g/kg habek was incorporated in the diet. This outcome and the result of organoleptic would lead to conclude that habek may be used safely with broilers, provided that more studies are needed to determine the effect of active ingredients of the herb on the performance and immunity of the birds.

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