

Effect of dietary supplementation of crushed seed of Celery (*Apium graveolens*) on Blood traits & Some Immunological parameters of broiler roosters (Hubbard flex).

تأثير الإضافة الغذائية لمسحوق بذور الكرفس (*Apium graveolens*) في صفات الدم وبعض المعايير المناعية لذكور فروج اللحم (هبرد فلक्स).

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Abstract

Targeted research study the effect of adding crushed seed of Celery (*Apium graveolens*) on blood traits & some immunological parameters of broiler roosters (Hubbard flex). Thirty roosters, Twenty-six weeks age, were randomly divided into three equal groups. First group (control) fed on the standard provender along the experimental period (7 weeks), second & third groups (T₁ & T₂) fed on standard provender supplemented with 2.5 & 5 kg/ton, respectively. At the end of experiment, blood samples were obtained from wing vein for estimation of white blood cells counts, differential leukocytes counts, phagocytic activity for neutrophils and monocytes. Total protein, globulin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP) enzymes, IgG, IgA & IgM concentrations in blood serum were calculated. The result showed that crushed seed of Celery (*Apium graveolens*) supplementation in the two treated groups caused a significant increase (P<0.05) in white blood cells counts, Lymphocytes, Monocytes & Phagocytic activity when compared with control group. At the same time, the result of total protein & globulin showed a significant increase (P<0.05) in two treated groups compared with control. The result of (AST, ALT & ALP) enzymes showed a significant decrease (P<0.05) in two treated groups compared with control. At the same time, the result of IgG & IgA concentrations showed a significant increase (P<0.05) in the both T₁ and T₂ groups compared with control.

Key words: Celery seed, Blood traits & Immunological parameters, Broiler.

الخلاصة

استهدف البحث دراسة تأثير إضافة مسحوق بذور الكرفس (*Apium graveolens*) في بعض الجوانب الفسلجية والمناعية لذكور سلالة فروج اللحم. استخدم 30 ديكاً من سلالة فروج اللحم هبرد فلक्स بعمر 26 أسبوعاً، وقسمت عشوائياً إلى ثلاث مجموعات بواقع 10 طيور لكل مجموعة: مجموعة السيطرة (C) تناولت العليقة الأساسية خالية من أي إضافة، ومجموعتي المعاملة الأولى والثانية (T₁ و T₂) تناولت العليقة الأساسية مضافاً إليها بذور الكرفس بقيمة 2.5 و 5 كغم/طن علف، على التوالي. وفي نهاية مدة التجربة، أخذت نماذج دم من الوريد الجناحي لغرض قياس المعايير الدموية (العدد الكلي والتفريقي لخلايا الدم البيض)، فضلاً على اختبار كفاءة عملية البلعمة للخلايا العدلة والوحيدة وبعدها تم الحصول على مصل الدم وقياس مستوى البروتين والكلوبيولين وALP وALT وAST وIgM وIgA وIgG. أظهرت النتائج وجود ارتفاع معنوي (P<0.05) في العدد الكلي لخلايا الدم البيض والخلايا اللمفية والوحيدة ومعدل الخلايا البلعية في مجموعتي المعاملة مقارنة بمجموعة السيطرة في نهاية التجربة. كما أشارت نتائج الدراسة إلى إن الإضافة الغذائية لمسحوق البذور في المجموعتين T₁ و T₂ أدت إلى حصول ارتفاع معنوي (P<0.05) في تركيز البروتين الكلي والكلوبيولين في مجموعتي المعاملة مقارنة بمجموعة السيطرة، كما أظهرت النتائج حدوث انخفاض معنوي (P<0.05) في مستوى الإنزيمات ALP وALT وAST في مجموعتي المعاملة مقارنة بمجموعة السيطرة في نهاية التجربة. من جانب آخر، أشارت النتائج إلى ارتفاع مستوى تركيز IgG & IgA في مصل الدم معنوياً (P<0.05) في كل مجموعات التجربة الثلاث.

Introduction

During the past 50 years, the growth rate of broiler chickens has been improved greatly. Feeding antibiotics as growth promoters had a substantial role in poultry industry. The World Health Organization (WHO) has recently identified antibiotic resistance as a major problem for public health on a global scale (1 and 2). The medical plants and their product including plant extract or essential oils are introduced as candidates or in broiler diets in which their beneficial effects as pathogenic feed additive have been proven (3). It has been reported that medical plants and herbs contain a wide variety of active phytochemicals including the flavonoids, terpenoids, lignans, linalool, and essential oil (4).

In general, the use of various parts of plant and materials as dietary supplements may positively affect poultry healthy, productively and immune response specially (5). Celery seed (*Apium graveolens*, family *Apiaceae*) containing powerful healing factor and active component in response to investigations by researchers seeking to explain some of the medicinal used as antibacterial, anti-inflammatory, condiment, carminative, diuretic and for treatment of bronchitis, asthma, rheumatism, arthritis, urinary calculi, constipation as well as liver and spleen disorders (6, 7 and 8). The active isolated compounds from the celery seed and its oil was Delta limonene, B-Selinene, Flavonoids (Apiin, Apigenin), Sesquiterpene, Phthalide, 3-n-butylphthalide, Sedanoide, fatty acids as Linoleic acid, Oleic acid, Myristic, Palmitic, Petroselinic. as well as Volatile oils & Amino acids (tyrosine, glutamine) (9 and 10). The isolated compound from seed flavonoids (Apigenin) have important role for preventing coagulation and aggregation of platelets in blood vessels (11). A compound known as 3-n-butylphthalide (3nB) was discovered as the active component of celery have medical effects including lowering of Blood pressure & cholesterol by its ability to block B receptors in blood vessels causing vasodilatation (12). However, no or limit reports are available on the effect of crushed seed of celery on poultry health. The aim of the present experiment was to study the effect of crushed seed of celery (*Apium graveolens*) on some physiological and immunological parameters of broiler roosters.

Materials and Methods

Seed of celery (*Apium graveolens*) were obtained from local market of herbs and medicinal plants, these seed were grinded in an electrical blender into a fine powder which was packed in air-tight bags plastic till use for basal diet supplementation. All experimental analysis of this study were carried out at Department of Animal Resource/ College of Agriculture/ University of Kerbala. During the period 26/1/2013 to 30/3/2013. A thirty roosters of broiler (Hubbard flex), Twenty-six weeks age, were randomly divided into three groups of ten animals in each group. The experimental diets were control (C) fed on the standard provender along the experimental period (7 weeks), second group (T₁) fed on standard provender supplemented with 2.5 kg/ton third group (T₂) fed on standard provender supplemented with 5 kg/ton.

At the end of experimental period, blood sample were taken from wing vein, and divided into two parts, 1st part 1.5 ml put into Ethylene Diamine Tetracetic Acid (EDTA) tubes for estimation of white blood cells counts according to (13), differential leukocytes counts according to (14), phagocytic activity for neutrophils and monocytes were determined according to (15). 2nd part 3.5 ml put in tubes without anticoagulant, separation of serum was carried out by centrifugation of the blood at 3000 rpm for 10 min. the clear serum was transferred carefully to clean and dry vials and kept in deep freezer -20 until analysis for determination of total serum protein according to (16), After albumin concentration was determined, then globulin concentration was calculated by:

$$\text{Globulin concentration (g/100ml)} = \text{total protein concentration (g/100ml)} - \text{Albumin concentration (g/100ml)}$$

Aspartate & Alanine aminotransferase (AST & ALT) enzymes were determined according to (17) & Alkaline Phosphatase (ALP) enzyme according to (18). Serum IgG, IgA, & IgM concentration were measured by using Nephelometry method according to (19).

Statistical analysis

All the values are expressed as mean± SD. Data of the experiment were analyzed using one way analysis of variance (ANOVA 1). Least significant difference (LSD) was carried out to estimate the significance of difference between individual groups. P value less than 0.05 was considered significant (20).

Results

The result of the effect of crushed celery seed (*Apium graveolens*) on white blood cell (WBC) count, differential leukocytes counts, Phagocytic activity for neutrophils and monocytes of broiler roosters were illustrated in table 1. The result indicated that there is a significant increased (P<0.05) in the WBC, Lymphocytes, Monocytes & Phagocytic activity in two treated groups compared with the control group. while the increment in Hetrophils & Acidophils didn't reach the significant level (P>0.05)in the comparison between the three groups.

Table 1: Effect of crushed celery seed (*Apium graveolens*) on hematological parameters of broiler roosters.

Groups Parameters	C	T ₁	T ₂
WBC(10 ⁹ /L)	21.45 ±0.459 c	25.39 ±0.1 b	28.828±0.056 a
Hetrophils %	24.53±0.179 a	25.91 ±0.35 a	28.02 ±0.166 a
Acidophils %	1.738 ±0.059 a	1.865 ±0.019 a	2.124 ±0.035 a
Lymphocytes %	60.16±0.024 c	62.296±0.079 b	64.6±0.034 a
Monocytes %	4.854±0.042 c	5.489±0.022 b	6.231±0.029 a
Phagocytic activity %	62.464±0.09 c	66.65 ±0.14 b	70.244 ±0.042 a

*Values given as mean ± SD.

*The different letters within the same rows are significant differences at P<0.05.

Table 2 showed the effect of crushed celery seed on total serum protein, globulin, (AST, ALT& ALP) enzymes of broiler roosters. The results are shown that crushed seed of Celery (*Apium graveolens*) supplementation in the two treated groups caused a significant increase (P<0.05) total serum protein and globulin when compared with control group. On the other hand, AST, ALT &ALP concentration, revealed a significant decrease (P<0.05) in the two treated groups compared with control.

Table 2: Effect of crushed celery seed (*Apium graveolens*) on biochemical parameters of broiler roosters.

Groups Parameters	C	T ₁	T ₂
Total protein (g/100ml)	4.18 ±0.021 c	4.56 ±0.011 b	5.4 ±0.029 a
Globulin (g/100ml)	2.35±0.017 c	2.67 ±0.026 b	3.45 ±0.061 a
AST (IU/mol)	35.504 ±0.105 a	28.37 ±0.059 b	23.598 ±0.111 c
ALT (IU/mol)	11.26±0.039 a	9.45 ±0.074 b	7.59 ±0.045 c
ALP (IU/mol)	81.99 ±0.401 a	78.38 ±0.479 b	60.28 ±0.215 c

*Values given as mean ± SD.

*The different letters within the same rows are significant differences at P<0.05.

The result of the effect of crushed celery seed (*Apium graveolens*) on immunoglobulin (IgG, IgA & IgM) of broiler roosters were illustrated in table 3. The result indicated that there is a significant increased in the IgG & IgA in two treated groups compared with the control group. while the increment in IgM didn't reach the significant level ($P>0.05$) in the comparison between the three groups.

Table 3: Effect of crushed celery seed (*Apium graveolens*) on immunoglobulin (g/L) parameters of broiler roosters.

Groups Parameters	C	T₁	T₂
IgG	2.623 ±0.018 c	3.386 ±0.033 b	3.978 ±0.009 a
IgA	2.108±0.029 c	2.642 ±0.011 b	2.846 ±0.008 a
IgM	1.933 ±0.006 a	1.955 ±0.016 a	2.099 ±0.042 a

*Values given as mean ± SD.

*The different letters within the same rows are significant differences at $P<0.05$.

Discussion

The result of this study revealed that celery seed (*Apium graveolens*) supplementation to male broiler in a dose of 2.5 & 5 kg/ton for 7 weeks produced beneficial effects on bird healthy and improvement immunity. the positive effects of celery seed characterized by increased WBC, lymphocytes, Monocytes, Phagocytic activity & immunoglobulin concentration. These effects may be mediated through several mechanisms since the seed itself is a complex mixture of many biologically active compounds. Phytochemicals analysis of the plant showed that (*Apium graveolens*) is contained vitamin B6, B1, B2, A, calcium, magnesium, phosphorus, tocopherol and iron (21). Also celery seed was an excellent source of vitamin C which is a known immune system booster and strong antioxidant, The detoxification effect of vitamin C is manifested by removal or minimization of free radicals (22). That could improved the cellular and humoral immunity through enhancement the functions of lymphocytes, monocytes & heterophils by increased the proliferation of lymphocytes, T & B-cells then production of immunoglobulins (23, 24 and 25). In Addition, immunoglobulin secretion is a complex metabolic process playing an important role in the process of the immune system to fend off antigen. The results of present study showed increase in concentration of serum immunoglobulin, lymphocytes & Phagocytic activity which lead to increase released cytokine, all these changes could regulate and enhance the immune functions that provide health benefits for combating disease challenges (26). As well as, (27) mentioned that ethanolic extract of celery seed 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. Also (28) reported that celery seed had antimicrobial effect against wide range of bacteria which improves the general health conditions of animal that may be reflected in increased immune response.

on the other hand, the flavonoids can display a huge array of biochemical and pharmacological effects that affect the function of the immune system and inflammatory processes (29). Due to their antioxidant, antiviral, anti-allergic & anti-inflammatory activity, they are considered "health -promoting, disease- preventing dietary compounds"(30). Positive effects of flavonoids on immune responsiveness might have a variety of underlying mechanisms. Activated immune cells generate free-radicals and increase oxidative stress(31 and 32), while T-and B-cell-based immune reactions are highly sensitive to oxidative damage to oxidative damage(33). Flavonoids can reduce oxidative stress by directly scavenging free - radicals, by interfering with free-radical producing mechanisms and by increasing the function of endogenous antioxidant(34). Additionally, *in vitro*, flavonoids may have profound direct effects on a variety of immune and

inflammatory cell functions, particularly once cells are activated(29) with inhibitory as well as stimulatory actions(35). on the other hand, celery seed have antioxidant and anti-inflammatory effects (36), and these are related to inhibition of prostaglandin synthesis as an anti immune substance and resulting better humoral response(37).

Our result shows the positive effect of crushed celery seed (*Apium graveolens*) on serum biochemistry of broiler breeder. The increase of total protein concentration in 5 kg/ton celery seed came in agreement with pervious report using crush seed of celery (*Apium graveolens*) and Ginger *Zingiber officinale* on blood traits of broiler breeder hens.(38). The elevation in total protein due to celery seed (*Apium graveolens*) supplementation groups could due to the biological active compounds of celery (*Apium graveolens*) seed particularly glycosides, which have stimulating effects for building proteins and prevent break down it (39). The present study revealed that the celery (*Apium graveolens*) seed has no harmful effect on liver function in bird. Our findings are in agreement with those obtained by (36), who observed decreased in AST, ALT & ALP enzymes and increased GSH content in the liver of rats treated with n-butanol extract of celery (*Apium graveolens*) seed when compared to control and diabetic rats. The decreased in AST, ALT & ALP enzymes might due to the biochemical compounds of celery seed as (D-carvon, D-limonen & myrcen), which have biological effects on aminotransferase enzymes and important role for elevation the antioxidant enzymes (Sorbitol dehydrogenase & Glutamic dehydrogenase) (40, 41 and 42). Our results are in agreement with those obtained by (38) who observed decreased in AST, ALT & ALP enzymes and increased GSH & MDH content in the liver of broiler breeder hens treated with celery (*Apium graveolens*) seed & Ginger (*Zingiber officinale*) when compared to control. Finally, the antioxidant activity of celery seed may also play an important role in cell protection from enhancement of oxidative injuries, also the ability of (*Apium graveolens*) for improvement immunity comes from these important Vitamin, biochemical compounds & Minerals in there components .

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