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Impact Laying Times of Broiler Breeder Supplement with Aromatic Oils (Miarom) in drinking water on Hatchability, Maternal immunity and serum antioxidant and antioxidant Statuses hatched chicks

ABSTRACT

Adding aromatic oils (Miarom) in drinking water of broiler breeder was evaluated experimentally of its effect on hatchability percentage, immunity, Lipid profile, and biochemical traits at different laying times (10:00 AM and 02:00 PM). A total of 3600 eggs of broiler breeder (Ross-308) at age 60 weeks, were collected and divided into 5 treatments of different times of egg collection and laying times as follows: (T1: General Control, T2: Control 10:00 AM, T3: Control 02:00 PM, T4: Miarom 10:00 AM and T5: Miarom 02:00 PM). Results indicated the used Miarom from Miavit company - Germany (this extract contains: 200 grams essential oils mixture such as eucalyptus oil, anise oil, thyme oil, and peppermint oil) per litter it added in drinking water 1ml/10L for 7 days. The results of this study showed ($p \leq 0.01$) increased in hatching traits percentage, chick's antibody titer against Newcastle Disease (ND) and Infectious Bronchitis Virus (IBV) by ELISA, high-density lipoprotein HDL, total protein and glutathione GSH-Px antioxidant enzyme. Also, significantly ($p \leq 0.01$) reduced infertile eggs, triglycerides TG, total cholesterol TCH, low-density lipoprotein LDL, corticosterone hormone, and malondialdehyde antioxidant enzyme. In conclusion, results showed that aromatic oils supplementation in broiler breeders leads to improve hatchability and immune status at different laying times, noticed that both Miarom treatments had a positive effect on studied parameters and T4 10:00 AM laying time was most effective in all parameters of the study.

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INTRODUCTION

Poultry industry is one of the main agricultural subsectors worldwide (El-Hack *et al.*, 2020). Several antibiotic growth promoters in past had been used in poultry feed aimed to prevent disease, increase some useful microorganisms in intestinal microflora and improve growth performance (Krishan and Narang, 2014). also, feed additives are used for broiler breeders to increase utilization of the limited feed allowance and in turn, improve egg production performance, fertility and hatchability (KAZEMI *et al.*, 2013). Researchers are now focusing on alternatives in place of antibiotics because of the emergence of bio resistance for this, spices, plant extracts, and herbs received increasing attention (Krishan and Narang, 2014).

The use of natural feed additives as substitutes for antibiotic in poultry production has become an area of great interest, herbs or medicinal plants consists of many pharmacologically active

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chemical compounds which have antioxidant, antimicrobial, antiviral, antifungal and anti-inflammatory effects as well as immune-modulatory properties (Mustafa, 2019). The positive effects of dietary essential oil on feed conversion, egg production performance, immunity, eggshell quality and egg weight has been confirmed in layer hens by previous studies (Puvača *et al.*, 2020). Most studies that investigated the effects of essential oil (EO) on biochemical blood parameters illustrated valuable impacts of EO on parameters, particularly its effects on antioxidant activity, lipid profile and immunity (El-Hack *et al.*, 2020; Alagawany *et al.*, 2021).

(Miarom-L) containing a mix of anise, thyme, peppermint and eucalyptus oils with vitamins B complex, AD3E, vitamin E and selenium) it's an effective material for maintaining the safety of the respiratory system, it is used after giving vaccines and in cases of viral and bacterial respiratory infections, the product also effectively stimulates the appetite of birds, in addition to raising immunity and controlling heat stress, at different laying time on reproductive characteristics of broiler breeder by measuring egg quality and hatching traits. Furthermore, the effect of Miarom on the immunological response of post-hatching chick. (Seyidoglu *et al.*, 2019). Thus, this study aims to the impact of laying time hatchability and immune status of broiler breeder supplements with aromatic oils.

METHODS AND MATERIALS

This study was carried out in TaqTaq poultry breeder farm and Hatchery project / Kosar company/Koya-Erbil, the trial started on 13/10/2021 to 17/11/2021. A completely randomized design with 3600 eggs of broiler breeder (Ross-308) at age 60 weeks, were collected and divided into 5 treatments at different times of egg collection and laying times as follows: (T1: General Control, T2: Control 10:00 AM, T3: Control 02:00 PM, T4: Miarom 10:00 AM and T5: Miarom 02:00 PM) used to evaluate the effect of adding 1ml Miarom to 10 L water for 7 days at different laying times. The farm temperature was controlled under 22-29°C, broiler breeder was exposed to a 16-h photoperiod a long an experimental period. Drinking water available *ad libitum* and feed consumption presented according to Ross 308 broiler breeder guide. The production ratio of broiler breeders was formulated according to the NRC (Council, 1994). Hatchability %, immunity, Lipid profile and biochemical trait at different laying times (10:00 AM and 02:00 PM) were calculated throughout the experiment.

At age 60 weeks 3600 eggs were taken from each treatment in both Miarom and control at different laying times and were set for 18 days in an incubator at (37.8°C) and 60% humidity then transferred to the hatchery for 3 days at (37.25°C) and 70% humidity until the end of 21 days of hatching. At the end of hatching process all chicks (live and dead) were counted, hatchability, infertile eggs, fertile egg, hatching of fertility eggs and hatching of all eggs were determined. Blood was collected from the chicks at age 4 days, and then centrifuged to obtain serum to measure the antibody titer of (ND) and (IBV) by direct ELISA Synbiotics (Biocheck – ELX 800). Total cholesterol and triglycerides were determined using same reagent kits for serum analysis and atherogenic index calculated as ratio of LDL/HDL. Plasma total protein was detected according to Biuret technique using a private kit (RANDOX) by spectrophotometric technique was used to determine plasma total protein, corticosterone hormone was measured by ELISA microplate reader (MRX® II Dynex Technologies, USA), malondialdehyde and glutathione peroxidase were determined. All data analyzed by using Complete Randomize Design (CRD) by SAS (SAS, 2005) and significant differences among treatment means determined by multiple Duncan's range tests at level 0.01 (Duncan, 1955).

RESULTS AND DISCUSSIONS

The results in table (1) shows percentage hatching traits eggs of broiler breeder dietary addition with Miarom in drinking water at different laying times. Infertile eggs had significantly ($P \leq 0.01$) decrease in all treatments compared with general control especially observed great decrease in T4 (10:00 AM Miarom), compared with control treatments. Percentage of each fertility, hatching fertile eggs and hatching total eggs significantly ($p \leq 0.01$) increased in all treatments with Miarom compare with the control treatments, and highest percentage was in T4 compared to controls. The same results were also found by Soliman *et al.*, (2016), the decreases of Malondialdehyde in the treatments of Miarom supplementation in (Table 3) may causes improvement in hatching traits. Also,

both El-Hindawy *et al.*, (2021) used oregano, and Liu *et al.*, (2021) founds that *Bacillus subtilis* and essential oil (BSEO) had better fertility, hatchability, and hatchability fertile eggs in broiler breeders during the late laying period. While results in another study showed that different dietary levels of mixture essential oils from anise, thyme, fennel, rosemary and black cumin no significant effected on hatchability of fertile eggs, fertility and hatchability of set eggs (Olgun *et al.*, 2014).

Table (1): Impact laying time on percentage of hatching traits of broiler breeder supplement with aromatic oils (Miarom)

Treatments	Time of egg collection	Hatching traits (%)			
		Infertile eggs	Fertility	Hatching of fertile eggs	Hatching of total eggs
T1	-	13.34±1.17 ^a	86.66±4.03 ^c	83.67±3.68 ^c	78.33±3.23 ^c
T2	10:00 AM	10.29±0.93 ^b	89.71±3.91 ^b	88.06±3.47 ^b	84.04±2.95 ^b
T3	02:00 PM	9.92±0.81 ^b	90.08±3.77 ^b	85.56±4.02 ^b	83.33±3.03 ^b
T4	10:00 AM	4.95±0.33 ^c	95.05±3.05 ^a	96.79±3.15 ^a	91.09±2.88 ^a
T5	02:00 PM	9.38±0.72 ^b	90.62±3.50 ^b	95.72±3.09 ^{ab}	86.50±3.10 ^{ab}
S. L		**	**	**	**

T1: general control, T2: control 10:00 AM, T3: control 02:00 PM, T4 Miarom 10:00 AM, T5 Miarom 02:00 PM. ^{a,b,c} different superscripts within rows for same trait significant differences at $P \leq 0.05$. **Means each factor and their interaction followed by same letters not significantly different from each others, according to multiple Duncan's ranges test at level (0.01).

The result in Figure (1) illustrated antibody titer against (ND) and (IBV). Results show positive effect of aromatic oil (Miarom) on chicks' immunity stimulate significantly ($p \leq 0.01$) increases in antibody responses against (ND) and (IBV) in both different laying times (10:00 AM and 02:00 PM) with Miarom had higher antibody responses which significantly ($p \leq 0.01$) increased compare with other treatments. This consistent with previous studies, which demonstrated dietary supplementation aromatic oils enhanced specific immunity responses in laying hens and broilers.

Also, the two control treatments had a significantly ($p \leq 0.05$) increase compared with the general control which has lower antibody titers. Also, T4 had a great increase in anti-bodies titer against both (ND) and (IBV) when compared with all other treatments. These results show that aromatic oil supplementation in water tends to increase immunity. In addition, essential oils (peppermint and eucalyptus) had positive effect against (ND), compared to their control treatment ($P \leq 0.05$) (Awaad *et al.*, 2016). On the other hand, herb and cinnamon mix infusion significantly improve immunity response to NDV in comparison to control and received only turmeric infusion (Sadeghi *et al.*, 2012). Consumption of 0.5 and 1.0% of mixtures thyme and mentha caused increased antibody in Japanese quail (Abasi and Daneshyar, 2020). While, no effect of dietary treatment on serum (IB) and (ND) titers detected for laying hens fed same mixture essential oil of six herbs at end of 16 weeks and 58 weeks, feeding period respectively (Özek *et al.*, 2011; Bozkurt *et al.*, 2012).

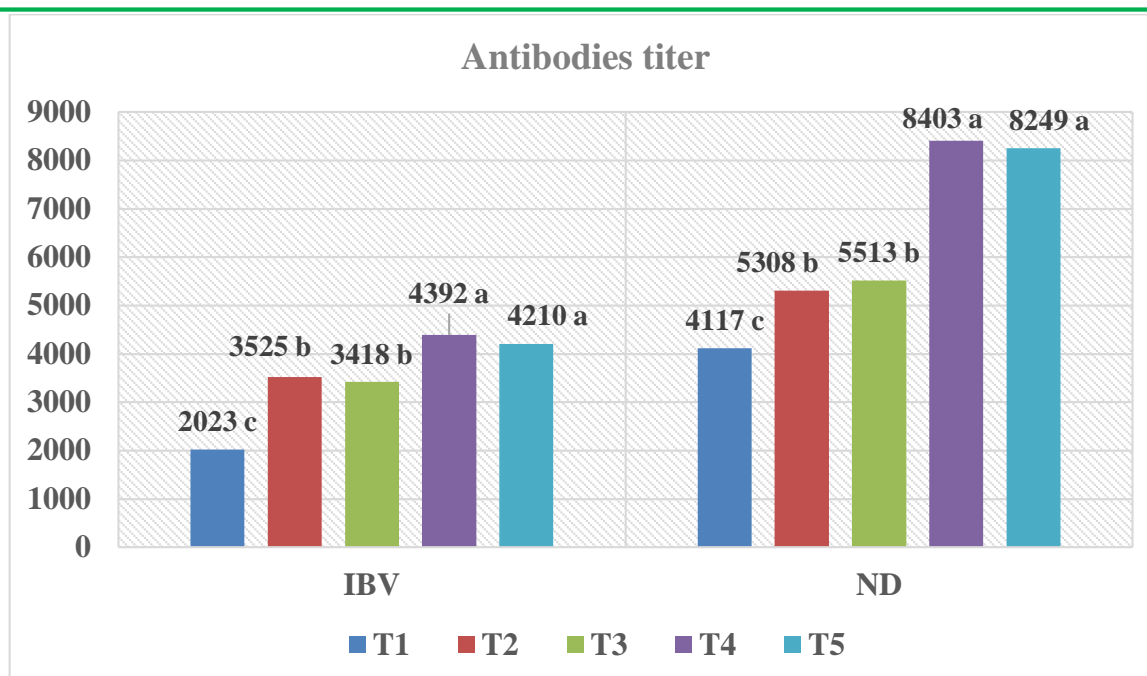


Figure (1): Impact laying time on antibodies titer of broiler breeder supplement with aromatic oils (Miarom)

Add T1: general control, T2: control 10:00 AM, T3: control 02:00 PM, T4 Miarom 10:00 AM, T5 Miarom 02:00 PM.

The results in Table (2) shows lipid profile in serum of hatched chicks' dietary addition with Miarom in drinking water at different laying time. The data showed that triglycerides (TG), total cholesterol (TCH) and low density lipoprotein (LDL) had significantly ($P \leq 0.01$) decreased in all treatments compared with general control especially had a great decrease in T4. On the other hand, the results of high-density lipoprotein (HDL) significantly ($p \leq 0.01$) increased in all treatments with Miarom compared with the control treatments, and T4 increased higher than T5 and other treatments.

The results agreement with Radwan Nadia *et al.*, (2008) who found some EO (Rosemary and Curcuma longa) decreased both (TCH) and (LDL-ch) compared to control. These result partially agreement with previous find of Soliman *et al.*, (2016) adding the oregano to laying hens' diets decreased (TCH), (HDL-ch), (LDL-ch) and (TG) fractions compared to the untreated control group.

Table (2): Impact laying time on Lipid profile in serum of hatched chicks of broiler breeder supplement with aromatic oils (Miarom)

Treatments	Time of egg collection	Lipid profile (mg/100 ml)			
		TG	TCH	LDL	HDL
T1	-	355±23.7 ^{ab}	178±18.5 ^{ab}	146.5±16.3 ^{ab}	31.5±4.6 ^{cd}
T2	AM	301±20.9 ^b	164±15.8 ^b	129.4±10.8 ^b	34.6±3.9 ^c
T3	PM	378±22.5 ^a	192±20.2 ^a	165.0±17.0 ^a	27.0±2.5 ^d
T4	AM	251±19.4 ^d	128±11.9 ^c	85.9±7.5 ^d	42.1±3.1 ^a
T5	PM	289±20.3 ^c	147±13.5 ^{bc}	108.6±9.3 ^c	38.4±2.9 ^b
S.L		**	**	**	**

T1: general control, T2: control 10:00 AM, T3: control 02:00 PM, T4 Miarom 10:00 AM, T5 Miarom 02:00 PM. ^{ab,c} different superscripts within rows for same trait significant differences at $P \leq 0.05$. **Means each factor and their interaction followed by same letters not significantly different from each others, according to multiple Duncan's ranges test at level (0.01). Triglycerides: TG, total cholesterol: TCH, low density lipoprotein: LDL, high density lipoprotein: HDL.

The results in table (3) shows some serum biochemical of hatched chicks' dietary addition with Miarom in drinking water at different laying time. Total protein (g/dl) significant ($p \leq 0.01$) increase in all treatments compared to general control and T4 treatment had a great increase compared with

the other treatments. These findings agree with Mustafa, (2019) who indicated that results of total protein significant ($p \leq 0.05$) increase in eggs of broiler breeder dietary addition with garlimmune extract in drinking water at different laying times. Also, the results of Corticosterone hormone (ng/ml) and Antioxidant enzymes of MDA (nmol/ml) were significantly ($p \leq 0.01$) decreased in all treatments especially had a great decrease in T4 which has a lower percentage of Corticosterone hormone (ng/ml) and MDA compare with other treatments.

The studies reported that antioxidant bioactive compound of aromatic plants such as rosemary and thyme can use to effected in MDA affecting lipid peroxidation in blood. Some studies revealed decrease MDA concentration after application of essential oil herbals. Eucalyptus aromatic oil's inclusion reduced serum MDA linearly ($P < 0.05$), in an experiment on broiler chicken (Mohebodini *et al.*, 2021). Similar findings have been noted by Mousavi *et al.*, (2017) which found addition of 200 mg/kg mixture aromatic oil of laying hen caused decrease plasma MDA concentration. These agree with Lipiński *et al.*, (2019) investigate decrease in MDA concentration when adding essential oil herbal in Ross 308 broiler chickens. On the other hand, dietary inclusion of thyme essential oil at levels 150 and 200 mg/kg decreased serum concentration of cholesterol and corticosterone in broiler chicks investigate by (Rafat Khafar *et al.*, 2019).

While Antioxidant enzymes of Glutathione peroxidase GSH-Px (U/ml) significant ($p \leq 0.01$) increased in Miarom treatments and T4 was the best. Another study obtained that oregano essential oil improved ($P \leq 0.05$) activity of glutathione peroxidase in birds (Zhang *et al.*, 2021). Also, the results in agreement with Noruzi *et al.*, (2022) indicated Thyme essential oil as a dietary supplemental has a favorable effect on blood (GSH-Px) activity.

Table (3): Impact laying time on some serum biochemical of hatched chicks of broiler breeder supplement with aromatic oils (Miarom)

Treat-ments	Time of egg collection	Total protein (g/dl)	Corticosterone hormone (ng/ml)	Antioxidant enzymes	
				MDA (nmol/ ml)	GSH-Px (U/ml)
T1	-	2.68±0.157 ^c	26.33±2.7 ^{ab}	10.63±0.63 ^{ab}	7.91±0.58 ^c
T2	10:00 AM	3.07±0.166 ^b	23.71±2.9 ^b	8.82±0.61 ^b	8.11±0.52 ^c
T3	02:00 PM	2.82±0.130 ^{bc}	30.05±2.5 ^a	11.95±0.75 ^a	6.47±0.40 ^d
T4	10:00 AM	3.85±0.193 ^a	13.90±1.4 ^d	3.75±0.44 ^d	13.75±0.72 ^a
T5	02:00 PM	3.12±0.202 ^b	17.45±1.8 ^c	5.68±0.49 ^c	11.34±0.76 ^b
S.L		**	**	**	**

T1: general control, T2: control 10:00 AM, T3: control 02:00 PM, T4 Miarom 10:00 AM, T5 Miarom 02:00 PM. ^{ab,c} different superscripts within rows for same trait significant differences at $P \leq 0.05$. **Means each factor and their interaction followed by same letters not significantly different from each others, according to multiple Duncan's ranges test at level (0.01). MDA: Malondialdehyde, GSH-Px: Glutathione peroxidase.

DISCUSSION

The beneficial addition of Miarom in broiler breeder drinking water at different laying times (10:00 AM and 02:00 PM) was positively affected on hatching traits observed improvements in fertility, hatching of fertile eggs, hatching of total eggs, total protein, HDL, antioxidant enzymes and increase immune against (ND) (IBV) by ELISA also, reduced infertile eggs, Corticosterone hormone (ng/ml), TG, TCH and LDL. In the present study, the adding of Miarom at 10:00 AM in broiler breeder drinking water improved in the percentage of hatching traits, the concentrations of antioxidant enzymes, Lipid profile and immune response of hatched chicks compared with the treatments of adding Miarom at 02:00 PM laying time.

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تأثير وقت وضع البيض لأمهات فروج اللحم بإضافة الزيوت العطرية (Miarom) في ماء الشرب على الفقس والمناعة الأمية ومضادات الأكسدة في مصلى الأفراخ الفاقسة

نضال عبدالغني مصطفى

زيهه مصلح مجيد

قسم الثروة الحيوانية/ كلية الزراعة/ جامعة صلاح الدين- أربيل/ العراق

الخلاصة

أختبر تجريبياً تأثير إضافة الزيوت العطرية Miarom في ماء الشرب لأمهات فروج اللحم في نسبة الفقس، المناعة، نسبة الدهون والصفات البيوكيميائية في أوقات مختلفة من وضع البيض (10:00ص، 02:00م). أشارت النتائج إلى أن استخدام Miarom حيث يحتوي كل لتر على المواد التالية: (200 مل من خليط الزيوت العطرية مثل زيت الأوكالبتوس وزيت الينسون وزيت الزعتر وزيت النعناع) في ماء الشرب بتركيز 100 مل / 1000 لتر لمدة 7 أيام، أدى بشكل ملحوظ ($p \leq 0.01$) إلى تحسين صفات الفقس، ورفع مستوى الأجسام المضادة للكتاكتيت ضد مرض نيوكاسل (ND) وفيروس التهاب الشعب الهوائية المعدية (IBV)، البروتين الدهني عالي الكثافة HDL، البروتين الكلي وإنزيم الجلوتاثيون GSH-Px المضاد للأكسدة. أيضاً، خفضت بشكل ملحوظ ($p \leq 0.01$) البيض الغير مخصب، والدهون الثلاثية TG، والكوليسترول الكلي TCH، والبروتين الدهني منخفض الكثافة LDL، وهرمون الكورتيكوستيرون، وإنزيم malondialdehyde المضاد للأكسدة. حيث لوحظ أن كلا معاملي Miarom كان لهما تأثير إيجابي على المتغيرات المدروسة وأن الوقت وضع البيض صباحاً T4 10:00 كان أكثر فاعلية في جميع الصفات المدروسة.

الكلمات المفتاحية:

Miarom، فقس، مناعة و وقت وضع البيض (صباحا و مساء).