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Synergistic Impacts of Effective Microorganism and Thermal Stress on Growth Performance, Haematological Parameters and Heat Shock Protein in Quails

M. M. Younes[1] , Shahbaa K. Al-Taee[2] and H.B. Al-Sabaawy[3]

[1]*Agriculture Directorate, Iraq.*

[2] *Department of Pathology and Poultry Diseases, Collage of Veterinary Medicine, Iraq.*

[3]*Department of Pathology and Poultry Diseases, Collage of Veterinary Medicine, [1]University of Mosul, Iraq.*

Abstract

THIS study aims to determine the effect of effective microorganism utilizing on growth performance, haematological parameters and heat shock protein in quails exposed to heat stress. A one days aged birds were used for 45 days of the experiment. The experiment were executed by comparing the control group with other three groups (heat group for 8hr thermal at 37-38 Celsius daily from 07 am:00-1pm,Effective Microorganism group treated with drinking water 1000ppm dose and the fourth groups treated with effective microorganism and exposed to heat stress) .quails weight measurements were carried out during 7,14,21,28 and 35 days in addition to body weight gain ,haemoglobin ,packed cell volume and heat shock proteins .The collected data was analysed by one and two way ANOVA test utilizing statistical social science (SPSS).The finding show that effective microorganism had highly significant effects at $p \leq 0.05$ on growth performance and blood parameters ,while heat shock proteins level showed no significant interaction expect the heat group and during the last week of the experiment. The result refer that Effective microorganism might be a main effective factor for chicken production to reduce thermal stress,eventually protects the chicken industry ,particularly in hot spot environment areas.

Keywords: Synergistic, Effective microorganism, Quails, Heat shock proteins.

Introduction

Quail is a diversified poultry bird that is raised for the purpose of producing eggs and meat commercially. These birds are characterized by unique characteristics such as rapid growth, high egg production rate, resistant to diseases, and short generation and incubation period, which makes them suitable for animal agriculture. Due to their low weight, size, and space requirements, quail rearing can be started with little investment capital compared to raising ducks and chickens for the purpose of producing eggs and meat[1].Effective microorganism are a culture of living microorganisms consisting of fungi and various types of bacteria such as (photosynthetic and lactic acid bacteria). Its beneficial effects appear through its ability to suppress the growth of bacteria that cause disease and aid in the digestive process [2]. It was used for the first time in 1986 at Ryukus University in Japan. In the 1990s, they were widely used in breeding fields. The use of these organisms in poultry feed helped improve meat quality, eliminate unpleasant odors, reduce toxicity, prevent intestinal pathogens, improve the balance of intestinal microbes and increase weight gain [3]. According to[4] EM using in broilers may improve their immunity, performance ,and parameters of intestine; however, more research is necessary to fully understand how EM works. Heat stress is considered one of the main problems in poultry farming fields, which negatively affects the productive, behavioural, immune traits and the reproductive rate many factors that act as a defence mechanism against high temperatures [5&6]**.** Damage inside the cell can be reduced by restoring homeostasis. Several indicators for blood picture that could be used to evaluate the function of the oxygen carrying capacity of the blood flow [7] such as total count of red blood corpuscles, level of hemoglobin, packed cell volume (PCV). Other factors have been exhibited to have an impact on

*Corresponding author: H.B. Al-Sabaawy, E-mail: hadeelbasim2006@gmail.com, Tel. 07705598182
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the blood analysis criteria[8] if the animal has had an exposure to them for example: sex, age, environmental conditions as nervous tension and weather conditions [9&10] with heat stress occurrence gene expression changes. Among these mechanisms is the activation of about 500 genes during the first minutes of exposure to heat stress [11], including the heat shock factor and heat shock protein, which is considered a means of defence against heat hyperpyrexia[12]. Slawinska *et al*. [2020] demonstrated that exposure of broiler to 32 temperature decreased digestion of food due to reducing blood flow. This work aimed to assess the effects of heat stress and effective microorganism on blood parameters, growth performance and heat shock proteins [14].

Material and methods

Ethics Approval

All techniques process in this work were acknowledged by the care of the animal welfare committee , university of Mosul, college of veterinary medicine (code No.UM.VET.2023.033)

Animals experiment

A total of 80 quail's birds were used. The birds were incubated for 10 days, after which the experiment began and continued for 45 days. The animals were arranged at randomly in the cages with the size of 140-160g ,with commercial diet the quails were feeding ,exploratory animals were split into four groups(20)birds per group (control group, heat group 6 hr\days, effective microorganism(EM) group with 1000ppm dose in drinking water [17] and the four group treated with the both heat and EM. Three animals were randomly selected from each group, and a blood sample was taken via the brachialis vein at 7,14,21,28 and 35 days, blood specimen were taken with two minutes following the initial distribution(removal of birds from the cage) then collected into heparin -tubes ,stored in a cooled icebox and send to the lab for determination of haemoglobin concentration and packed cell volume(%). Body weight and body weight gain were recorded at 7,14,21,28 and 35 days of the experiment .Body weight gain was calculated based on the body weight in the current time period, minus the weight in the previous time period according to[15].

Heat Shock Protein Assay

Heat Shock Protein resoluteness was performed using a mercantile specific chicken kit (Code .No.201-16-033.) (Shanghai,China) according to the recommendation of the manufacture .All specimen were run in the same evaluate to prevent inter variability.

Analysis of Statistical Data

The different blood parameters, growth performance and heat shock proteins level in control and all groups were statistical analysis by statistic pack for social scientific science (SPSS).In the form of standard error mean ± and at $p \leq 0.01$ and $P \leq 0.05$ probability value[16] .

Results

Blood Criterion

Tables (1 and 2) displays the impacts of effective microorganism and thermal stress on Hb level that showed the significant variation and treatment means at in the first and fourth group at 28 day , while the week means was high at 21 and 35 day .A high significant variation at $P \leq 0.05$ in the 28 day of the fourth group and the treatments mean on the volume of the packed cell, while the week mean showed the high variation at 21,28 and 35 days.

TABLE 1. Effect of Treatments on Hb level /ml.

	Age/day					Treatments mean
	7	14	21	28	35	
Control	12.50 ± 0.50^{de}	12.50 ± 0.50^{de}	13.92 ± 0.24^{bc}	15.33 ± 0.58^{a}	14.67 ± 0.58^{ab}	13.78 ± 1.25^{a}
Heat	11.33 ± 0.35^{f}	9.93 ± 0.12^{g}	9.70 ± 0.52^{g}	9.00 ± 0.50^{g}	9.23 ± 0.68^{g}	9.84 ± 0.93^{c}
EM	11.43 ± 1.12^{f}	11.77 ± 0.40^{ef}	12.80 ± 0.80^{de}	9.83 ± 1.04^{g}	12.6 ± 0.60^{de}	11.69 ± 1.3^{b}
Heat+EM	13.00 ± 0.66^{cd}	12.53 ± 0.50^{de}	14.03 ± 0.06^{b}	15.67 ± 0.58^{a}	14.87 ± 0.23^{ab}	14.02 ± 1.26^{a}
Weeks mean	12.07 ± 0.96^{bc}	11.68 ± 1.16^{c}	12.61 ± 1.87^{a}	12.46 ± 3.25^{ab}	12.84 ± 2.41^{a}	

Significant differences between treated groups vertically represented by letters at ($P<0.05$).

TABLE 2. Effect of Treatments on PCV (%).

	Age/day					Treatments
	7	14	21	28	35	mean
Control	38.00±0.87^{fg}	38.00±1.73^{fg}	43.00±2.00^{cde}	46.67±2.89^{ab}	44.33±2.08^{bcd}	42±3.98^{b}
Heat	34.00±1.00^{h}	29.83±0.76^{i}	29.33±2.08^{i}	27.67±0.58^{i}	28.33±1.53^{i}	29.83±2.55^{d}
EM	35.00±1.00^{gh}	35.00±0.87^{gh}	38.00±2.00^{fg}	30.00±0.00^{i}	36.33±3.21^{gh}	34.87±3.15^{c}
Heat±EM	41.33±1.53^{def}	40.67±1.04^{ef}	44.00±1.73^{bcd}	47.67±3.51^{a}	45.00±1.73^{abc}	43.73±3.17^{a}
Weeks mean	37.08±3.14^{ab}	35.88±4.32^{b}	38.58±6.29^{a}	38.00±9.82^{a}	38.50±7.34^{a}	

Significant differences between treated groups vertically represented by letters at (P<0.05).

Table (3) showed the effects of heat and effective microorganism on BW of quails during the experiment. In the 1st weeks all groups showed high body weights while in the 2nd ,3rd ,4th and 5th weeks effective microorganism groups showed the high body weight in contrast to the other groups.

TABLE 3. Effect of Treatments on weekly body weight (g).

	Age/day				
	7	14	21	28	35
Control	48.93+3.7^{a}	105.58+1.38^{b}	145.67+13.53^{ab}	173.67+14.92^{ab}	209+37.04^{ab}
Heat	50.13+50^{a}	102.75+7.13^{b}	131.22+6.35^{bc}	155.67+12.86^{b}	166+14.11^{b}
Heat±EM	51.87+3.56^{a}	109.17+1.81^{ab}	128.78+3.56^{c}	165.5+20.22^{ab}	201.33+19.5^{ab}
EM	57.13+4.99^{a}	117.42+9.03^{a}	158.44+7.71^{a}	197.17+19.04^{a}	244.33+36.12^{a}

Significant differences between treated groups vertically represented by letters at (P<0.05).

Table (4) showed that weekly weight gain of quails within the 5 weeks period of work. At week one of the current experimented all groups have the highest WG, throughout the 4 weeks of the experiment effective microorganism groups have the heights WG while the control groups showed heights WG during the second week , heat and effective microorganism also showed heights WG during the 3 week of the study .

TABLE 4. Effect of treatments on weekly weight gain (g).

	Age/day				
	1	2	3	4	totaly
Control	56.65+2.33^{a}	40.08+1.45^{a}	28.22+2.34^{b}	38.67+2.02^{b}	163.4+3.49^{b}
Heat	52.62+4.49^{a}	28.47+0.99^{b}	24.44+1.17^{b}	10.33+0.58^{c}	115.87+10.43^{c}
Heat±EM	57.3+3.57^{a}	19.61+1.88^{c}	36.55+1.55^{a}	36.33+1.53^{b}	149.47+16.03^{b}
EM	60.28+6.53^{a}	41.03+3.92^{a}	37.39+2.98^{a}	47.17+2.93^{a}	187.2+15.31^{a}

Significant differences between treated groups vertically represented by letters at (P<0.05).

Table (5) showed the effects of treatment on the level of HSP ,no significant effects of the treatment on the level of heat shock protein expect at the third group and during the last week of the experiment .

TABLE 5. Effect of treatments on Heat Shock Proteins level (ng/ml).

	Age					Treatments
	7	14	21	28	35	(mean)
Control	4.76±3.05^{b}	7.44±1.08^{ab}	9.15±2.68^{ab}	8.42±0.44^{ab}	3.78±3.23^{b}	6.71±2.95^{a}
Heat	3.86±2.45^{b}	5.04±2.47^{b}	7.97±0.51^{ab}	7.03±1.68^{ab}	11.10±8.48^{a}	7.00±4.38^{a}
EM	5.16±0.69^{b}	4.43±2.64^{b}	6.22±2.98^{ab}	7.64±0.67^{ab}	6.30±1.20^{ab}	5.95±1.97^{a}
Heat±EM	6.79±0.55^{ab}	8.50±0.57^{ab}	5.04±3.85^{b}	6.87±0.51^{ab}	6.30±1.57^{ab}	6.70±1.98^{a}
Weeks mean	5.14±2.04^{a}	6.35±2.39^{a}	7.09±2.89^{a}	7.49±1.04^{a}	6.87±4.83^{a}	

Significant differences between treated groups vertically represented by letters at (P<0.05).

Discussion

Ecological stressor ,including cold stress and heat stress are accountable for reducing health condition ,wellbeing and production attached with undesirable variety meat quality in addition color ,holding capacity of water ,and soft, egest breast muscle[18] in poultry increasing the beneficial effects of bacteria in the digestive system has shown vowing in increasing the performance of growth and reducing the negative effects of stress factor ,through safe guarding welfare of poultry and

industry economic gains when chicken exposure to heat stress. Increasing red blood cells contributes to improving the defence mechanism diseases [19].In cytokinin-producing birds, blood cells act as a defense system, and their number is affected by the severity of their composition. Tissue oxygenation is one of the factors affecting the formation of red blood cells. If the percentage of oxygen transported to organs and tissues decreases, these causes an increase in the production of erythrocyte. Hematological parameters in the control group were higher than those in the heat stress group as indicated in table 1 and 2. Factor of hem dilution, an adaptive reactive responses that allow loss of water by evaporation without affecting on volume of plasma may be the cause of a great reduction in Hb and PCV. While increasing in the atmosphere temperature during the summer could be the reasons in an increasing body birds consumption temperature of oxygen and respiration [19].Regard to the influence of effective microorganism on hematological parameters investigated in the current experiment, statistical significant variation increase in the haemoglobline and packed cell volume of quails and these relate to number of bacteria and type of it ,effective microorganism used in the work gave a good response to the blood physiology condition so that it could be used to be a good chickens health circumstances. Result of the current work showed that there is increasing in the body weights and body weight gain of quail through the experimental periods. However ,the effective microorganism groups showing significant increase in BW and BWG in contrast to all others groups and these result agreement with [19&20] who mention that broiler body weight gain was increased by L acidophilus usage or by a strain of Enterococcus Faecium or by a mixture of Lactobacillus spp[21]**.**Thermal stress is a major problem in broiler industry affecting growth performances of it, the body weight and body weight gain of the stressed group was less than other groups and theses result agreement with [22] who mention reduced in broilers and indigenous growth performances under heat stress (36-37 Celsius/ for six h per day) and these relate to restricted the intake of feed during thermal challenge .Heat shock protein are a proteins family, voiced in all living organisms under stress factors, HSP level is an indicator to thermal stress and the capacity of stress tolerance. These proteins are categorized into four family (HSP100,HSP90,HSP70 and HSP60)[23], HSP acts to promote the folding of proteins (Misfold &nascent) in stress conditions these proteins protects the cell and tissue from damage through enhancing the expression of proteins [24] HSP70 production is promoted through thermal stress in various organs like heart, liver, leukocytes and brain [25].furthermore HSP70 protect the epithelium of the gastrointestinal tract ,facilitated the absorption of nutrition and metabolism of it, HSP 70 up-regulate in acute condition of thermal stress (thirty days at 36 Celsius for three h)[26].This offers that thermal stress may increase the expression of HSP in chicken ., [27] mention that early heat did not effects on the expression of HSP at 36 Celsius for 1 h /day during 1-21 days, breed might play an important roles in revealing variable data ,resulted data could contributed to differences of breeds, collection of sample ,thermal treatment ecological condition.Thus, ,the impacts of thermal resistance on early exposure of heat may be dependent on environment and animals breeds .HSP may elevated by stress in chickens [26] recorded low expression of HSP70 in the liver, brain, and kidneys of early heat-treated (5-d-old, 36°C, 24 h) chicks after chronic heat stress (22–42 d, 35°C, 6 h/d) without early heat treatment. These variable results could be related to differences in the broiler breeds, sample collections, heat treatments exposure, and environmental conditions dependent. Rresults of the current study indicate that the use of Effective microorganisms in the drinking water of quails exposed to thermal stress had good impacts on the health status of the animal.

Conclusion

In the present study, effective microorganism significantly increased weekly body weight, body weights gain, haemoglobin concentration, packed cell volume and even heat shock proteins in chickens exposed to thermal stress. However, our data concluded that broiler diet supplementing with EM improving growth performance and blood parameters even during stress condition .in order to protect production of broiler from the negative impacts of stress factor we suggestion to adding effective microorganism as administration strategy.

Writers' contribution

All writers equally participated in equipping this work.

Interest collision

No collision of interest about this task.

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التأثيرات التآزرية للكائنات الحية الدقيقة الفعالة والإجهاد الحراري على أداء النمو ومؤشرات الدم وبروتين الصدمة الحرارية في طائر السمان

مروة ميسر ذنون ، شهباء خليل الطائي و هديل باسم السبعاوي

مديرية زراعة الانبار- فرع الامراض وامراض الدواجن - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.

الخلاصة

تهدف الدراسة الحالية الى تحديد تاثير استخدام المتعضيات الحية على اداء النمو والمعايير الدموية وبروتين الصدمة الحرارية لطائر السمان المعرض للاجهاد الحراري. تم استخدام الطيور بعمر يوم واحد ولمدة 45 يوما ،نفذت التجربة من خلال مقارنة مجموعة السيطرة مع ثلاثة مجاميع تمثلت (بالمجموعة المعرضة للاجهاد الحراري ولمدة 8 ساعات متواصلة ما بين 37 -38 درجة مئوية ومن الساعة السابعة صباحا ولغاية الساعة الواحدة ظهرا ،والمجموعة الثانية عوملت بالمتعضيات الحية وبجرعة 1000 جزء بالمليون بماء الشرب ،وتمثلت المجموعة الرابعة بالمجموعة المعرضة للإجهاد الحراري والمعاملة بالمتعضيات الحية ،تم قياس وزن جسم طائر السمان خلال 7و14و21و28و35 يوما من التجربة بالإضافة الى قياس معدل الزيادة الوزنية وقياس هيموجلوبين الدم وحجم الخلايا المرصوصة وبروتين الصدمة الحرارية تم تحليل البيانات باستخدام اختبار ANOVA وبمسار واحد ومسارين وببرنامج (SPSS). اظهرت النتائج وجود فرق معنوي عند مستوى الاحتمالية$p\leq0.0$ في معايير النمو والمعايير الدموية بينما لم يظهر بروتين الصدمة الحرارية اي فرق معنوي ملحوظ ما عدى المجموعة المعاملة بالحرارة وخلال الاسبوع الاخير من التجربة، نستنتج من نتائج الدراسة الحالية الى ان المتعضيات الحية قد تكون اداة ادارية رئيسة في صناعة الدواجن للحد من الاثار السلبية للإجهاد الحراري، وربما حتى حماية ورفاهية وانتاج الدواجن، وخاصة في المناطق التي تعاني من الطقس الحار.

الكلمات المفتاحية: التازر ،المتعضيات الحية ،طائر السمان ،بروتين الصدمة الحرارية.