EFFECT OF CHRONIC HEAT STRESS ON BROILER CHICKS PERFORMANCE AND IMMUNE SYSTEM.

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ABSTRACT

The present study was carried out to evaluate the effect of exposure to chronic heat stress on broiler chick performance and immune system. Also, the effect of adding probiotics was evaluated. Our results revealed that chicks exposed to chronic heat stress showed significantly decreased final body weight at 6^{th} week of age 1601.67±27.44 grams (gms) as compared to control chicks that weighed 1816.67±8.33 gms, at the same age. While, their feed conversion ratio was 2.14±0.20 and in control group it was 2.08±0.21. Adding probiotics to control or heat stressed chicks improved their performance, as body weights at 6^{th} week were 1918.67±8.33 gms and 1765±17.56 gms in in both groups, respectively. Meanwhile, their feed conversion ratio decreased. There were negative impact of chronic heat stress on immune organs / body weight ratios (bursa, spleen and thymus) as they were significantly reduced. The lowest ratios were 0.15±0.26, 0.21±0.03 and 0.13±0.01, for bursa, spleen and thymus, respectively as compared to control which had ratios of 0.33±0.04, 0.39±0.25 and 0.32±0.06 at the same age. Heat stressed chicks showed increased H /L ratio, the highest ratio was 0.42±0.03 while in control it was 0.20±0.02 at the same age . Serum natural agglutinin response as expressed as Log_2 revealed significant reduction in heat stressed chicks 2.31±0.10 as compared to 3.11±0.10 in control. Adding probiotic to stressed or non-stressed chicks increased immune organs / body weight ratio as compared to heat stressed or control chicks. Meanwhile, serum natural agglutinin levels were significantly higher as compared to heat stressed chicks.

INTRODUCTION

Heat stress is one of the major constraints that confront poultry production especially during the hot humid summer season. Poultry is raised under intensive rearing which require suitable environmental and

manegmental condition. Broiler chickens are homoeothermic; they maintain their central body temperature within a slight range irrespective of ambient temperature. So, birds have a thermoneutral zone that should be described as being a range in the environmental temperature in which energy needs for thermoregulation is minimum and the net energy for production is maximum ***(Furlan & Macari, 2002).*** When prevailing temperatures rise above the comfort zone, they experience heat stress. A reduction in feed intake of broilers can be expected to occur, and this is usually accompanied by a decline in growth rate**,** live weight gain, and feed efficiency thus negatively influence the performance of broilers. Body weight gain reduced more than food intake, since part of metabolizable energy intake was used for heat dissipation, impairing feed conversion ***(Geraert et al., 1996)***.

High environmental temperature appears to affect the development of specific immune responses in chickens. It has been shown that continuous exposure to elevated temperatures reduces the immune response, circulating antibodies were depressed. Cell-mediated immunity has also been reported to be adversely affected by high environmental temperatures **(*Miller and Quereshi, 1991).***

One of the methods to alleviate the effect of high environmental temperature on the performance of broilers is dietary manipulations ***(Sahin, 2002)***. In this respect, probiotics are one of the main approaches that have a potential to reduce negative impacts of heat stress. Probiotics stimulate the immunity of the chickens in two ways either through migratin of flora from probiotic throughout the gut wall and multiply to a limited extent or antigen released by the dead organisms are absorbed and thus stimulate the immune system **(*Tache et al., 2001).***

The main objectives of the present study were to determine the effects of chronic heat stress on broiler chicks performance and immune systems, and the effect of adding probiotic to alleviate this condition.

MATERIAL & METHODS:

A- Experimental chicks:

A total of two hundreds one-day old Hubbard chicks were used. The chicks were weighed and randomly allocated to one of four treatment groups with fifteen birds each with similar average initial weight. Chicks were reared on deep litter, kept under 23 hours (hrs.). Light: 1 hour (hr.) darkness (D**)** and stocking density of 10 birds / m^2, from hatching till day 42. Feed and water were provided ad-libitum.

B-Treatment groups:

Experimental chicks were assigned to one of the following groups:

1-Control group: chicks were kept on brooding temperature started with 33°C then temperature was then decreased by 2 °C each week until it reached 20-22°C and fixed till the end of |cf1experiment. Chicks received basal diet without probiotics.

2- Control group with probiotic: Chicks were kept under the same conditions of the control group and their diet was supplemented with probiotic (200 gm / ton) which consisted of *[Bacillus subtilis* spore concentrate (No. of spores 4 x 10^{12} CFU / kg), 0.5 %, Sodium alumino silicate 1.0 % and Wheat permeate 98.5 % as a Carrier].

3-Chronic heat stressed chicks:

Chicks were kept in controlled environmental chamber with dimensions of 1.80 x 2.40 x 2.34 m. Chamber was provided with thermostatically electric heater and small electric fan for the circulation of the air, two holes of 16 cm diameter were made to provide ventilation. Chicks were subjected to temperature of *(*33±2 °C*)* from day 1 till the end of the experiment, Chicks received basal diet without probiotics.

4- Chronic heat stressed chicks:

Chicks were kept in controlled environmental chamber and kept on the same temperature regime as chronic heat stress group and received basal diet supplemented with probiotic.

I- Effect of chronic heat stress on broiler performance and immune organs:-

At 2nd, 4th and 6th weeks of age, five chicks from each group were randomly selected and body weight was recorded then sacrificed. Blood samples were collected in clean and dry centrifuge tubes, all viscera were removed carefully by hand, and then carcass weight was recorded.

The percentage of weight of organs (Bursa, thymus and spleen) were calculated according to ***Jin et al., (1998)*** as the following:

$$\frac{\text{Weight of organ (gm)}}{\text{Carcass weight (gm)}} \times 100$$

The FCR was calculated according to ***Wagner at al., (1983)*** as following:

$$\text{FCR} = \frac{\text{Feed intake (gm) in a given period}}{\text{Body weight gain (gm) in the same period}}$$

Mortality rate was estimated according to ***Halpin (1975)*** by using the following formula:

$$\text{Mortality rate} = \frac{\text{Number of dead chicks}}{\text{Total number of chicks}} \times 100$$

II- Effect of chronic heat stress on H / L ratio and serum natural agglutinin levels:-

At 2nd, 4th and 6th weeks of age, two drops of blood were collected from the brachial vein, and blood smears were made on duplicate glass slides. These smears were stained with Giemsa stain. One hundred leucocytes were counted on each slide including heterophils, lymphocytes The H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes. Both slides were counted and the means were calculated for each bird. ***(Gross and Siegel, 1983).***

At 6 weeks of age blood samples were collected from all experimental chicks to estimate levels of natural agglutinins. Antibodies were measured with a hemagglutination test according to ***Giambrone et al., (1978)*** as following : Rabbit blood samples was collected into Alsever's solution (dextrose 20.5 g, 7.9 g sodium citrate, and 4.2 g sodium chloride in 1 L water) by cardiac puncture. Phosphate buffered saline (PBS, 1%, pH 8.1) was used to wash the RRBCs twice and for resuspension. Complement in chicken serum was inactivated by incubating the serum in a hot water bath at 56 °C for 30 min. For the hemagglutination assay, chicken serum (50 mL) was aliquoted into round-bottom 96-well plates and serial two fold dilutions was prepared with PBS. Suspension of RRBC was added to each well and incubated 1 h at 37 °C, and examined for evidence of hemagglutination. Results were expressed as $\log_2$ values.

Statistical Analysis:

Statistical analysis was performed by usig Stat-View program version 4.53 (1992- 1997), USA.

Results and Discussion:-

Effect of heat stress on body weights and feed conversion of broiler chicks:-

As shown in Table (1) and figure (1), at 2nd , 4th and 6th weeks of age chronically heat stressed chicks revealed significantly reduced body weight 304.67±5.18 gms, 830±2.89 gms and 1601.67±27.44 gms, respectively as compared to control group 375±2.89 gms, 936.67±4.41 gms and 1816.67±8.33 gms, respectively.

Meanwhile adding probiotic to non stressed chicks significantly increased body weight 390±2.89 gms, 980±2.89 and 1918.33±9.28 gms as compared to control chicks.

Concerning the effect of heat stress on food conversion as shown in Table (2) it was ranging from 1.12±0.20 to 1.37 ±0.12, 1.48±0.22 to 1.64±0.28 and 2.02±0.14 to 2.14±0.20 at 2nd , 4th and 6th weeks of age, respectively. The lowest mean was recorded in control chicks that received probiotic.

Heat stressed chicks showed increased feed conversion, Meanwhile heat stressed chicks received probiotic in ration had lowered feed

conversion as compared to heat stressed chicks. On the other hand, adding probiotic decreased feed conversion as compared to control group.

The depression in the growth rate and body weight gain at high environmental temperature might be due to many factors which include decreasing feed consumption ***(Snedecor and Cochran, 1994)***, ineffeicient digestion. ***(Har et el., 2000),*** impaired metabolism ***(Farrell and Swain, 1978).*** At high ambient temperatures, there is a decrease in protein synthesis ***(Geraert et al., 1996),*** probably due to reduced plasma amino acid concentration and to lower energy supply ***(Temim et al., 2000).***

The obtained results agree with ***Suk and Washburn (1995)*** who have shown decreased efficiency of feed utilization with increased environmental temperatures, they assume that the decreased feed consumption observed in heat stressed birds is closely related to the extra heat load accumulated in the course of heat stress.

Non stressed chicks that received probiotics revealed significantly increased body weight as compared to control and had lower feed conversion ratio. Better broiler performance by adding probiotics could be attributed to that probiotic maintain a healthy balance of intestinal flora by producing organic compounds, such as lactic acid, hydrogen peroxide and acetic acid that increase the acidity of the intestine and inhibit the reproduction of many harmful bacteria ***(Rasic, 1983)***.

Effect of heat stress on mortality rate of broiler chicks:

Regarding the effect of heat stress on mortality rate Table (3) revealed that the mortality rates were higher in heat stressed chicks where they were 20%, 13.33 % at 2^{nd} and 4th as compared to control. Mortality rates at 6^{th} week were close to control which could be due to adaptation of chicks to stress.

Chicks recived probiotic either stressed or non stressed had reduced mortalities as compared to heat stressed and controls, while mortalities were 2% in control chicks received probiotic and 6.67% and zero% in heat stressed chicks received probioticat 2^{nd} and 4^{th} week. However at 6^{th} week no mortalities were recorded in both groups.

This result agreed with ***Abu-Dieyeh (2006)*** who stated that rearing broilers at high ambient temperatures 31-35°C caused a significant increase in mortality rate. ***Mashaly et al., (2004)*** reported that heat stress increased the percentage of mortality. This increase in mortality could be due to inhibition of immune responses.

Reduced mortality in chicks received probiotic could be explained in the light of reduction of colonization of enteropathogens in the gastrointestinaltract and invasion and this agree with that reported by ***Vicente et al., (2007).***

Effect of heat stress on on bursal index, thymus%, spleen % and H:L% in broiler chicks

Table (4) and **Figure (2)** showed that bursal index ranged from 0.15±0.26 to 0.34±0.18. 0.33±0.50 to 0.43±0.18 and 0.20±0.15 to 0.24±0.20 at 2nd, 4th and 6th weeks of age, respectively.

At 2nd and 4th weeks of age heat stressed birds showed significant decrease in bursal index while, in 6th week this decrease were not statistically different as compared to control . Adding probiotic to heat stressed group increased bursal index by 60% , 9.09 and 5% at 2nd , 4th, 6th weeks of age, respectively as compared to heat stressed group. At 2nd, 4th and 6th weeks of age, there was significant increase in bursal index in control group with probiotic as compared to control group. With respect to the effect of heat stress on thymus / body weight ratio it ranged from ٠,١٢±٠,٦٠ to 1.06±0.07. 0.31 ±0.03 to 0.47±0.05 and 0.21 ±0.03 to 0.44±0.05 at 2nd, 4th and 6th weeks of age, respectively. At 2nd, 4th and 6th week heat stressed birds showed significant decrease in thymus % as compared to control. Adding probiotic to heat stressed chicks seems to increase thymus % by 13.33, 16.33% and 80.95% as compared to heat stressed group at 2nd, 4th, 6th weeks of age, respectively. At 2nd, 4th, 6th weeks of age there was significant increase in thymus % in control group with probiotic as compared to control group.

Concerning the effect of heat stress on spleen % that it ranged from 0.13±0.01 to 0.35±0.02, 0.15±0.03 to 0.20±0.02 and 0.20±0.03 to 0.26±0.02 at 2nd, 4th and 6th weeks of age, respectively. At 2nd, 4th and 6th week heat stressed chicks showed significant decrease as compared to control group. However, adding probiotic increase spleen % by 84.61, 13.33 and 15% at 2nd, 4th, 6th weeks of age, respectively as compared to heat stress group. On the other hand, at 2nd and 4th week there was significant increase in spleen % in control group with probiotic as compared to control group. Obtained results agree with ***Puvadolpirod*** *and* ***Thaxton (2000)*** who reported that general regression of lymphoid organs is recognized as an important response of chickens to chronic stress. ***Bartlett and Smith (2003)*** reported that All organ weights (thymus, bursa, spleen,) were significantly reduced by stress. This could have been a result of the reduction in feed intake, thereby

providing less nutrients for the proper development of these organs.

Effect of heat stress on Hetrophil: Lymphocyte ratio (H / L):

Table (4) and **Figure (2)** show the effect of heat stress on H / L% which ranged from 0.15±0.01 to 0.42±0.03. 0.15±0.01 to 0.41 ±0.01 and 0.20±0.01 to 0.38±0.04 at 2^{nd} , 4^{th} and 6^{th} week of age, respectively. Heat stressed group showed significant increase in H: L ratio at 2^{nd}, 4^{th} and 6^{th} week while heat stress with probiotic showed increased trend at 2^{nd} week and revealed significant increase in H: L ratio at 4^{lh} week and increased trend at 6^{th} week. Adding probiotic to heat stressed chicks decrease H: L% by 47.62%, 39.02 % and 44.74 % as compared to heat stressed group.In general heat stress has adverse effect on immune system. However probiotics has improving effects. Decreased H / L ratio in probiotic treated groups could be due to stimmulation of immune system and reduction of inflammatory reaction of chicks **(*Simmering and Blaut, 2001)***. ***Borges et al., (2004)*** observed that heat stress altered the proportion of heterophils (increased) to lymphocytes (decreased) in blood.

Effect of heat stress on natural agglutinin response*:*

It was clear from Table (4) and figure (3) that heat stressed chicks showed a significant decrease in serum natural agglutinin level as expressed as $\log_2$ (2.31±0.10) as compared to control chicks (3.11±0.10). Adding probiotic to heat stressed chicks significantly increased natural agglutinin response (2.71±0.17) as compared to heat stressed chicks. Non stressed chicks that received probiotic showed the same natural agglutinin response as compared to control chicks. Obtained results revealed that heat stress had adverse effect on the level of natural agglutinin of chicks which indicate impairment in the humeral immune response , this agree with ***Belay and Teeter, (1994)*** *and* ***Bartlett and Smith (2003)*** who reported that heat stress has adverse effects on alterations in immune function and it impedes with disease resistance. Also heat stress showed suppressive reaction on humeral immune response of chickens ***(Ferket and Qureshi, 1992).***

Table (1): Effect of heat stress on weekly body weights in grams of broiler chicks

	Control	Control +probiotic.	Chronic heat stress group	Chronic heat stress group +probiotic
2nd week	375±2.89 [b]	390±2.89 [a]	304.67±5.18[d]	341.67±5.18[c]
4th week	936.67±4.41[b]	980±2.89[a]	830±289[d]	880±2.89[c]
6th week	1816.67±8.33[b]	1918.33±9.28[a]	1601.67±27.44[d]	1765±17.56[c]

*Means within column having different letters (a, b, ……..) are significantly different at p< 0.05.

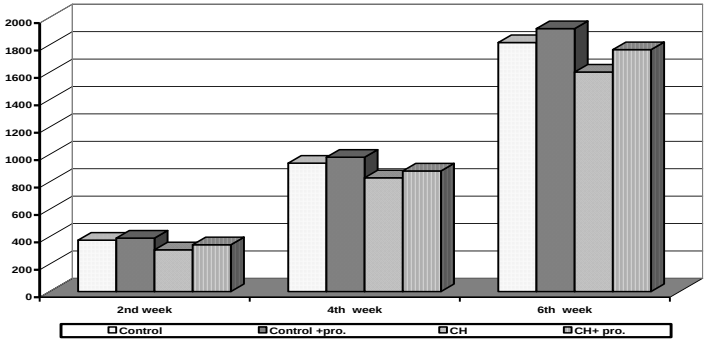

Figure (1): Effect of heat stress on weekly body weights of broiler chicks

Table (2): Effect of heat stress on food conversion of broiler chicks:

	Control	Control +probiotic.	Chronic heat stress group	Chronic heat stress group +probiotic
2nd week	1.34 ±0.23	1.12±0.20	1.37±0.12	1.30±0.25
4th week	1.64±0.28	1.48±0.22	1.59±0.15	1.62±0.12
6th week	2.08±0.21	2.02±0.14	2.14±0.20	2.05±0.15

*Means rows are not significantly different at p<0.05.

Table (3): Effect of heat stress on mortality rate of broiler chicks

	2nd week		4th week		6th week	
	No. of dead birds	Mortality %	No. of dead birds	Mortality %	No. of dead birds	Mortality %
Control	3	6	3	6	1	2
Control +probiotic.	1	2	1	2	0	0
Chronic heat stress group	6	20	4	13.33	1	3.33
Chronic heat stress group +probiotic	2	6.67	|charrsid11547747 0	0	0	0.00

*Means rows are not significantly different at p<0.05.

Table (4): Effect of heat stress on some immunological parameters in broiler chicks:

		Control	Control + probiotic.	Chronic heat stress group	Chronic heat stress group +probiotic
Bursal index	***2nd week***	0.33±0.04 [b]	0.34±0.18 [a]	0.15±0.26 [d]	0.24±0.39 [c]
	4th week	0.36±0.17 [b]	0.43±0.18 [a]	0.33±0.50 [d]	0.36±0.03 [c]
	6 ·af/th week	0.21±0.20 [b]	0.24±0.20 [a]	0.20±0.15 [b]	0.21±0.05 [b]
|aspalpha**Thymus (%)**	***2nd week***	0.79±0.08 [b]	1.06±0.07 [a]	0.60±0.12 [d]	0.68±0.05 [c]
	4th week	0.42±0.04 [b]	0.47±0.05 [a]	0.31±0.03 [d]	0.36±0.02 [c]
	6th week	0.39±0.25 [c]	0.44±0.05 [a]|fs22	0.21±0.03 [e]	0.38±0.04 [d]
Spleen (%)	***2nd week***	0.32±0.06 [b]	0.35±0.02 [a]	0.13±0.01 [d]	0.24±0.04 [c]
|brdrs	***4th week***	0.18±0.01 [b]	0.20±0.02 [a]	0.15±0.03 [c]	0.17±0.58 [b]
	6th week	0.24±0.01 [b]	0.26±0.02 [a]	0.20±0.03 [d]	0.23±0.01 [c]
H: L ratio	***2nd week***	0.20±0.02 [b]	0.15±0.01[b]	0.42±0.03 [a]	0.22±0.01[b]
	4th week	0.20±0.02 [c]	0.15±0.01[c]	0.41±0.01 [a]	0.25±0.01 [b]
	6th week	0.20±0.01 [b]	0.20±0.01[b]	0.38±0.04 [a]	0.21±0.01[b]
Natural agglutinin response	***6th week***	3.11±0.10 [a]	3.11±0.10 [a]	2.31±0.10[c]	2.71±0.17[b]

*Means within column having different letters (a, b, ……..) are significantly different at p<0.05.

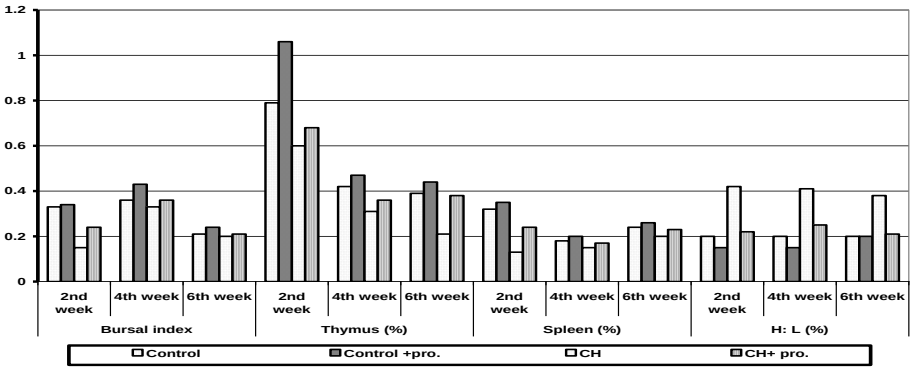

Figure (2): Effect of heat stress on bursal index, thymus%, spleen % and H:L% in broiler chicks.

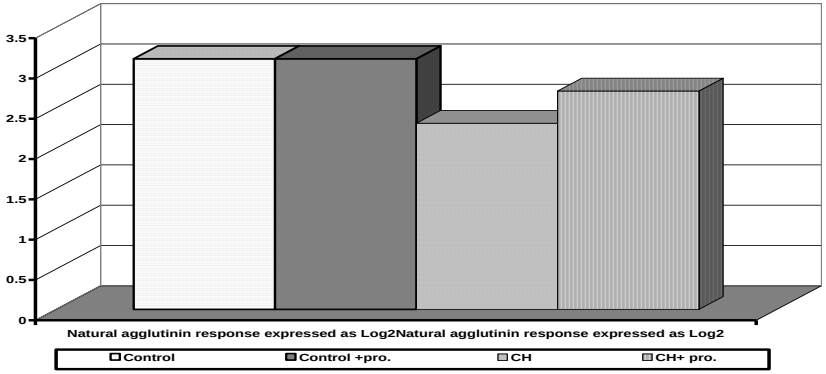

Figure (3): Effect of heat stress on serum natural agglutinin response.

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الملخص العربي

تأثير الإجهاد الحراري المزمن على أداء وجهاز المناعة لدجاج التسمين

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تم إجراء هذه الدراسة لاستبيان تأثير تعرض دجاج التسمين للإجهاد الحراري المزمن طوال فترة التربية وكذلك تأثير إضافة البروبيوتك على أداء الدجاج وكذلك الحالة المناعية. تم استخدام ٢٠٠ كتكوت عمر يوم تم تقسيمهم إلى أربعة مجموعات حيث تم تعريضهم لدرجة حرارة ٣٣ ±٢°م لمدة ٦ أسابيع وتم استخدام مجموعة ضابطة تعرضت لدرجات حرارة ٣٣ ±٢°م تم خفضها تدريجيا بمعدل ٢°م أسبوعيا حتى تصل ٢٠- ٢٢°م وتم دراسة تأثير إضافة البر وبيوتك على أداء الدواجن بالنسبة للمجموعة المعرضة للإجهاد الحراري وكذلك المجموعة الضابطة.

وجد أن الإجهاد الحراري كان له تأثير سلبي على أداء الدجاج حيث كان متوسط الأوزان فى الأسبوع السادس ١٦٠١,٦٧±٢٧,٤٤جرام مقارنة بالمجموعة الضابطة حيث كان متوسط الأوزان ١٨١٦,٦±٨,٣٣ جرام وكان معدل التحويل الغذائى٢,١٤± ٠,٢٠ أما فى المجموعة الضابطة كان

٢,٠٨ ±٠,٢١. إضافة البر وبيوتك للمجموعة المعرضة للإجهاد الحراري أدى إلى تحسين الأداء حيث ذاد معدل الأوزان فى الأسبوع السادس مقارنة بالمجموعة التي تعرضت للإجهاد الحراري حيث كان ١٧٦٥±١٧,٥٦جرام وكانت الزيادة معنوية فى كل الحالات. زادت نسبة النفوق بالنسبة للدجاج المعرض للإجهاد الحراري المزمن مقارنة بالمجموعات الأخرى.

وبدراسة تأثير الإجهاد الحراري على الجهاز المناعي وجد أن له تأثير سلبي على أوزان كل من غدة فابريشيا والغدة السيموزية وكذلك الطحال حيث قلت نسبة أوزان تلك الأعضاء مقارنة بوزن الطائر. وبقياس نسبة الهيتروفيل / الليمفوسيت فى الدم كان هناك زيادة معنوية فى حالة التعرض للإجهاد الحراري المزمن مقارنة بباقي المجموعات . وكذلك وجد أن نسبة التلازن التي تدل على كفاءة كتاكيت التسمين أقل فى حالة التعرض للإجهاد الحراري . وأدى استخدام البر وبيوتك إلى زيادة معنوية فى نسبة أوزان تلك الأعضاء مقارنة بالمجموعة التي لم تأخذ البروبيوتك كما زادت نسبة التلازن وقلت نسبة الهيتروفيل / الليمفوسيت فى الدم مما يؤكد دور البروبيوتك فى زيادة الحالة المناعية لكتاكيت التسمين .