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Differential Leucocyte Count and Total Colony Count Changes in Heat Stressed Broiler

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ABSTRACT

The present study aimed to explain the role of additives in alleviation of the negative effect of heat stress on differential leucocytes count, heterophil / lymphocytic ratio with especial studies on the microbial count of duodenum, jejunum, ileum and caecum. A total of 270 day-old (Ross308) broiler chickens randomly divided into 6 groups, which were kept under elevated temperature (34-36°C) and feed diet containing 1% Nigella Sativa (G_2) or a mixture of sodium bicarbonate and potassium chloride (0.3% KCL+ 0.5% $NaHCO_3$) (G_3) or 2% coriander seed (G_4) or 0.03% Acetylsalicylic acid (aspirin) (G_5) or 250 mg of Ascorbic acid (Vitamin C) (G_6) for 6 weeks. The previous parameters were recorded after slaughtering to take microbial sample and collecting blood parameters. The results explained that, there was a significant increase lymphocyte percentage in case of G_2, G_3, G_4, G_5 and G_6. while, there was significant decreases in Heterophil percentage, heterophil/lymphocyte ratio (H/L), eosinophils percentage, monocytes percentage, jejunum total colony count and caecum total colony in case of (G_2, G_3, G_4, G_5), (G_6, G_2, G_3, G_4, G_5), (G_6, G_3), (G_5; G_2), (G_3; G_2) and (G_2, G_4) respectively in compared with control group ($P<0.05$). It could be concluded that black seed and coriander seed have a positive effect on heat stress broiler diet.

Introduction

The domestic fowl is a homoeothermic, which can live comfortably only in a relatively narrow zone of thermo-neutrality extending from 14.5ºC to 25.5ºC (Freeman, 1969). Heat stress may occur when ambient temperature exceeds 32°C (Ahmed and Maghraby, 1995). So that, some physiological changes occur in broiler in Egypt during the summer season (Toyomizu *et al*., 2005)

Such as acute heat stress leads to decreased monocytes and lymphocytes proportions (Altan *et al*., 2000 a,b). While, birds exposed to chronic heat stress (4 weeks) had a lower white blood cells count and higher heterophil/lymphocyte ratio (H/L) (Mashaly *et al*., 2004), a 70 % decrease in the number of lymphocytes, increase about 2.5, 15, 20 and 44 % in esinophils, monocytes, basophiles, and heterophils respectively (Grey *et al*., 1989), reduced the number of heterophil, increased the numbers of basophils and monocytes in birds (Maxwell, 1993) and increased heterophil percent and reduced basophiles percent (Osman, 1996; Vijayan and Rema, 1997).

Heterophil/lymphocyte ratio is a sensitive indicator of heat stress in fowls, which increased significantly at the first and last days of heat stress compared to control group (Altan *et al*., 2000a; 2008, Nadia 2003; Gharib *et al*., 2005).This in-

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creasing measures a physiological change in organs such as a trophy of the bursa of Fabricius and thymus that is influenced by the effect of heterophils. (Aengwanich and Chinrasri, 2003).

On the other hand, the bad effect of the widely used antibiotics in broilers' diet that have a bad effect on broiler meat or production. So we try to use the previous feed additives to get rid of some bad effect of heat stress on broiler hematology with reference to their effect as an antimicrobial. One of the best methods used to control heat stress is the chemical management of acid-base balance by supplementing feed or water with different electrolyte salts as sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl), which led to a higher carcass weight and dressing percentage than the control. (Ahmad *et al*., 2005). Dietary sodium bicarbonate at 0.25%, 0.50% and potassium chloride at 0.2%, 0.3% supplementation alone and combination between them give good and positive effects on body weight, feed conversion of golden motazah hens (Hassan *et al*., 2011) increased feed consumption values of laying hen and gave the best values of feed conversion (EL-Sheikh and Salama, 2010), improved significantly the average values of body weight, weight gain, feed intake and feed conversion (Ghazalah *et al*., 1998).

Significant increase in broiler body weight was obtained in groups that fed diet contain 0.2 % of acetylsalicylic acid (Al-Obaidia and AL-Shadeedi, 2010), which improved the performance and physiological traits. (Abdel-Fattah, 2006) and improved feed conversion ratio (Hassan *et al*., 2003).

White Leghorn layers fed diets supplemented with vitamin C /kg diet during the hot summer season recorded a significantly higher body weight, increased feed intake and live weight gain than the control (Konka *et al*., 2009; Ajakaiye *et al*., 2010), beneficial effects on the performance and economics of production of broiler chicks exposed to heat stress. (Onu, 2009), improved the weight gain of birds (Sobayo *et al*., 2008), a slight increase in feed intake of the birds (Oruseibo and Alu, 2006) and a positive significant effect on final body weight and feed conversion (Metwally, 2005).

Finally, our result of previous work of adding 1% black seed or 2% coriander seed in diet of heat stress broiler for 6 weeks led to significant increases in feeding behavior, feed intake, average final body weights, average body weight gain and dressing percentage, and significant decreases in panting behavior, water to feed ratio, T_3 and corticosterone. Also, no significant difference was indicated in drinking behavior and T_4. In addition, there was numerically, but not significant decrease in feed conversion ratio and numerically, but a non-significant increase in slaughter and carcass weight. (Ramadan *et al*., 2014). The present study was undertaken to explain the role of these additives in alleviation the negative effect of heat stress on te differential leucocytes count, heterophil / lymphocytic ratio with especial reference to the microbial count in the duodenum, jejunum, ileum and caecum .

Materials and methods

Groups classifications

A total number of 270, one unsexed day old "Ross 308" broiler chicks reared in a laboratory animal house in the Veterinary Teaching Hospital at Faculty of Veterinary Medicine, Assiut University, Egypt. The chicks were divided into four groups each one consisted of 15 birds/ replicate (Table 1).

Table 1. Show the classification of different groups included in the experiment

Group name	Numbers	Diet
Control group (G_1)	45 birds (3 replicate each one 15 birds)	Basal diet only
Nigella sativa (black seed) (G_2)	45 birds	Basal diet + 1% Nigella sativa
Sodium bicarbonate and Potassium chloride (KCL+$NaHCO_3$) (G_3)	45 birds	Basal diet + (0.3% potassium chloride + 0.5% Sodium bicarbonates)
Coriander seed (G_4)	45 birds	Basal diet + 2% coriander seed
Acetylsalicylic acid (aspirin) (G_5)	45 birds	Basal diet + 0.03% Acetylsalicylic acid
Ascorbic acid (Vit. C) (G_6)	45 birds	Basal diet + 250mg of Vit. C

Management and cleaning

Day to day management carried out in keeping the facility clean. After daily cleaning, cleaned feeders and drinkers were dried and filled with food and water. Also, the contaminated wastes and dead birds were hygienically disposed by incineration.

Lighting

Continuous lighting program (23 hours lightning: 1 hour darkness) used. 60 watt bulb suspended 2.20 meters at head height of the birds.

Temperature and relative humidity

In the first 14 days of age, temperature and relative humidity of the room maintained according to the recommendations of broiler breeder company (Aviagen, 2002). Then the heat stress experiment (34°C) started from 14: 42 days old. The heat provided by using an electrical heater. Ventilation was done by electrical ventilator to remove any excess of ammonia gases or heat.

Ambient temperature determined by maximum and minimum thermometer and indoor relative humidity measured by using wall mount wet and dry bulb hygrometer. The readings were taken every day and the average of weekly readings was calculated (Fig. 1).

Diet and feeding

The basal diets formulated using NRC (1994) guidelines. It contained 21 proteins (starter type from 0 – 14 days) and 23% (grower type from 15 – 42 days) with 3200 kcal/kg.

The birds fed on antibiotic-free mash diet (containing thoroughly mixing treatment substance) ad libitum. Bird was given free access to fresh and clean water throughout the experimental period. The feed was offered daily and residual feed was measured weekly.

The composition and metabolizable energy value of the diet (physical and chemical) were mentioned in Tables 2 and 3.

Feed additives sources and the percentage used The feed additives such as black seed and coriander seed were purchased from commercial market while the vitamin C, aspirin, potassium chloride and sodium bicarbonate were purchased from the medical company, the percentage of the feed additives were used according to previous studies. These additives were mixed thoroughly with the diet.

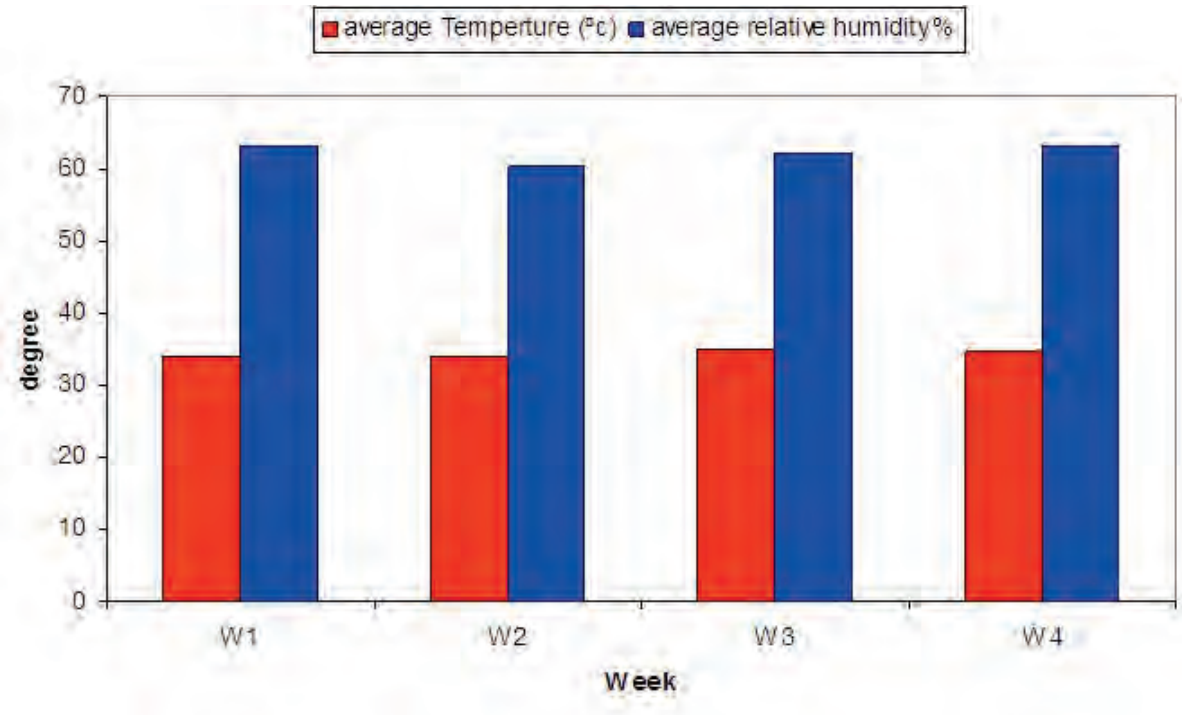

Fig. 1. Average temperature and average relative humidity during the experiment period

Table 2. Physical composition and metabolizable energy value of the diet

Ingredient (%)	Starter diet	Grower diet
Wheat	56.5	60
Soybean meal	33.5	29.3
Soybean oil	5.00	3.46
Palm fat	---	2.66
limestone	1.12	1.06
Mono Ca phosphate	0.08	1.46
Mineral premix	0.08	0.08
Cholin chloride	0.20	0.20
Methionine	0.20	0.17
L –Lysine- HCL	0.35	0.30
$NaCHO_3$	0.28	0.27
NaCL	0.10	0.35
Luprosil	0.40	0.53
Loxidan TD ^{100}	0.015	0.015
Vitamin premix	0.20	0.20

Table 3. Analyzed values of nutrients (%) in experimental diets

Nutrient (%)	Starter diet	Grower diet
Dry mater	88.8	89.2
Crude protein	23.1	21.1
Crude fat	6.89	7.71
Crude fiber	6.07	5.73
Crude ash	2.80	2.62
NFE	51	51.8
Methionine 2	0.5	1.24
Lysine 2	1.39	.45
Ca	0.98	0.92
P	0.84	0.68
ME (MJ/kg DM)	12.3	12.6

Birds' identifications

Bird identifications were carried by wing band.

Medication and vaccination

Medication was given to the experminted chicks according to the design in Table 4, while the vaccination program was done according to the design in Table 5.

Work items or measurements

Differential Leucocyte Count

At 42 days old, three blood smears from each treatment group, prepared immediately after slaughter and stained using Giemsa stain. A total of 100 white cells were counted by using an oil immersion lens of electrical microscope.
Heterophil / Lymphocyte (H/L) ratio: H/L ratio was calculated using the following equation:
H/L ratio = number of heterophil cells / number of lymphocyte cell.

Microbial counts

Intestinal contents from the duodenum, jejunum, ileum and caecum were evacuated and mixed immediately after slaughter, and then was stored separately in previously weighed screw-capped sterile plastic cups. The sealed containers were kept on ice until they transported to the laboratory for enumeration of microbial populations. One milliliter (1 ml) sample of each digesta was transferred into 9 ml of sterile normal saline and further dilutions were made. Aliquots (0.1 ml) of the appropriate dilutions of the serially diluted samples were then inoculated onto nutrient agar plates in duplicates.

The spread plate method using a sterile bent glass rod was used. Inoculated plates were incubated at 37°C for 24 hours, discrete colonies that developed on each plate were counted, and their

Table 4. Medication program that was applied during the experiment

Name of drug	Age	Dose of drug	Route of administration
Enroflox 10%	1 to 3 days of age	0.5 ml / liter	Drinking water
Royl colistin	1 to 3 days of age	1 g/ liter	Drinking water
Vitamins AD_3E	3 days / week	1 ml liter	Drinking water
Liquid minerals	3 days week	1 ml liter	Drinking water
Neo terramycin	4 to 10 days of age	3 g / liter	Drinking water

Table 5. Vaccination program that was applied during the experiment

Age of bird	Name of vaccine	Route of vaccination	Disease
6th days old	Hitchner-B1	Drinking water	Newcastle disease
17th days old	Lasota strain	Drinking water	Newcastle disease
29th days old	Lasota strain	Drinking water	Newcastle disease
12th days old.	Gumboro	Drinking water	Gumboro disease
23rd days old.	Gumboro	Drinking water	Gumboro disease

averages from two plates were calculated. Numbers of colony-forming units (cfu) expressed as log colony-forming units per gram of digesta content and expressed as cfu/mL (APHA, 2005).

Results

Differential leucocyte count

Data illustrated in Table (6) showed the effect of Nigella Sativa (G_2) or mix of sodium bicarbonate and potassium chloride (G_3) or coriander seed (G_4) or acetylsalicylic acid (G_5) or ascorbic acid (G_6) on differential leucocyte count and H\L ratio, the analysis of variance revealed that there were significant increases in lymphocyte percentages in cases of G_2, G_3, G_4, G_5 and G_6 compared with the control group. While, there were significant decreases in Heterophil percentage, H/L ratio, eosinophiles and monocytes percentages in cases of (G_2, G_3, G_4, G_5 and G_6), (G_2, G_3, G_4, G_5 and G_6), (G_3 and G_5) and (G_2 and G_3) respectively. Moreover, there were numerical, but not significance decreases in eosinophiles, monocytes and basophils percentages in (G_2 and G_6), (G_6) and (G_2, G_3, G_4, G_5 and G_6) respectively as well as, a numerical but not significant increases in cases of the eosinophiles and monocytes percentages in G4 and (G_4 and G_5) respectively compared with the control group.

Microbial total colony count

Results shown in Table (7) revealed that the effect of black seed, sodium bicarbonate plus potassium chloride, coriander seed, acetylsalicylic acid and vitamin C on the total colony count. Where, the mean values of duodenum total colony count of treatment substances were 8.68, 9.31, 9.06, 9.93 and 8.58 respectively, while that of control group was 9.12 CFU x 10^3 / ml. Moreover, the mean values of the ileum total colony count of treatment substances were 8.37, 9.12, 8.90, 9.00 and 10.21 CFU x 10^3/ ml, respectively, while it was 10.40 CFU x 10^3/ ml for the control group. At the same time, the mean values of the jejunum total colony count of treatment substances were 6.57, 9.27, 9.11, 9.27 and 9.51 CFU x 10^3 / ml, respectively, while that for the control group was 10.7 CFU x 10^3 / ml. Finally the mean values of caecal total colony count of treatment substances were 6.66, 9.86, 7.83, 9.41 and 10.85 CFU x 10^3 \ ml, respectively, while that for the control group was 10.52 CFU x 10^3 / ml.

Biostatistics of the previously mentioned data showed that, there was no significant difference in the duodenum and ileum total colony count of treatment groups and control one, however, there was a significant decrease in jejunum total colony count among black seed treatment and control group. While there was a significant decrease in

Table 6. Effect of black seed, sodium bicarbonate plus potassium chloride, Coriander seed, acetylsalicylic acid and vitamin C on Differential Leucocytes Count (%) and H/L ratio.

	Heterophil	Lymphocyte	Monocytes	Esenophiles	Basophils	H/L Ratio
Control	50.33 ± 0.88^{a}	34.33 ± 1.52^{e}	10.00 ± 1.15^{a}	4.33 ± 0.88^{a}	1.0 ± 00	1.37 ± 0.11^{a}
Black seed	20.33 ± 0.67^{c}	70.67 ± 9.02^{b}	$5.33\pm0.88^{b,c}$	$3.33\pm1.20^{a,b}$	0.33 ± 0.33	0.37 ± 0.05^{c}
Sodium bicarbonate – potassium chloride	12.67 ± 1.45^{d}	85.00 ± 5.0^{a}	2.00 ± 1.15^{c}	1.00 ± 0.58^{c}	0.67 ± 0.33	0.17 ± 0.03^{d}
Coriander seeds	22.00 ± 2.89^{c}	$62.33\pm2.52^{b,c}$	10.33 ± 1.14^{a}	4.67 ± 0.88^{a}	0.67 ± 0.33	0.37 ± 0.0^{c}
Acetylsalicylic acid	40.00 ± 2.08^{b}	49.33 ± 5.51	10.33 ± 0.88^{a}	0.67 ± 0.33^{c}	0.33 ± 0.33	0.68 ± 0.07^{b}
Vitamin C	35.00 ± 2.88^{b}	$54.00\pm5.00^{c,d}$	$7.67\pm1.5^{a,b}$	$2.67\pm1.45^{a,b}$	0.67 ± 0.33	0.64 ± 0.04^{b}

Table 7. Effect of black seed, sodium bicarbonate plus potassium chloride, Coriander seed, acetylsalicylic acid and vitamin C on Total colony count of broiler. (CFU x 10^3 / ml)

	Total colony counts			
	Duodenum	Jejunum	Ileum	Caecum
Control	9.12±0.44	10.7 ±0.4^{a}	10.40±0.99	10.52± 0.95^{a}
Black seed	8.68± 0.85	6.57±0.71^{b}	8.37±1.45	6.66 ±0.33^{c}
Sodium bicarbonate + potassium chloride	9.31±0.53	9.27± 0.70^{a}	9.12± 0.49	9.86 ±0.20^{a,b}
Coriander seeds	9.06 ±0.56	9.11± 0.15^{a}	8.90± 0.25	7.83±1.06^{b,c}
Acetylsalicylic acid	9.93 ±0.95	9.27± 1.05^{a}	9.00± 0.56	9.41± 0.56^{a,b}
Vitamin C	8.58 ±0.18	9.51 ±0.64^{a}	10.21±0.93	10.85 ± 0.44^{a}

caecal total colony count in black seed and coriander seed groups compared with the control group.

Discussion

The effect of heat stress on increasing heterophil percentage, H/L ratio, eosinophiles percentage, monocytes percentage and basophils percentage was previously studied by Grey *et al*. (1989); Maxwell (1993); Vijayan and Rema (1997); Altan *et al*. (2000a); Nadia (2003); Gharib *et al*. (2005); Gharib *et al*. (2008). Moreover, the effect of heat stress on decreasing lymphocyte percentage was studied by Altan *et al*. (2000 a,b).This result was disagreed with the present data which explained the importance to use the different treatments in alleviation of negative effect of heat stress on differential leukocyte count.

The decreasing heterophils percentages may be related to the ability of the treatment substances to relieve the effect of heat stress, which decreases the corticosterone secretion by sequence increase the immunity through increasing the lymphocyte numbers (Satterlee *et al*., 1989). While, increasing of lymphocyte percentage may be due to the immune stimulant of that substance and increasing the effect on lymphoid organs weight, especially with black seed and coriander seed. Moreover, it may be due to the role of vitamin C in the synthesis of leukocytes (Null, 2001). Meanwhile, decreasing the effect of acetylsalicylic acid on of H/L was agreed with previous data of Abou-El-Soud *et al*. (2006). This result may be due to the depressing effect on corticosterone concentration, which cause increased H/L ratio (Aengwanich and Chinrasri 2003). Moreover, the effect of Vitamin C to decrease H/L was agreed with the study of Satterlee *et al*. (1989); Zulkifli *et al*. (2000). This result may be related to the ability of the treatment substances in decreasing heterophils percentage and increasing lymphocytes percentage as mention before.

The caecum is one of the areas of greatest microbial activities in the gastrointestinal tract of chickens, and thus, can be described as the location for a very complex microbial ecosystem. Relative to other parts of the gastrointestinal tract, the caecum provides a stable environment for microorganisms, resulting in a large microbial population due to the slower transit time. In addition to that, intestinal microflora plays an important role in the health status of host animals. In general, intestinal bacteria may be divided into species that exert either harmful (pathogenic) or beneficial effects on host health. Therefore, a common approach to maintain host health is to increase the number of desirable bacteria in order to inhibit colonization of invading pathogens (Guo *et al*., 2004).

The applity of black seed to decrease the microbial count may be due to the anti microbial activity of the black seed, which were studied by Lee *et al*. (2003); Guo *et al*. (2004); Gilani *et al*. (2004); Abu-Dieyeh and Abu-Darwish (2008). As known Nigella sativa contains different components such as thymoquinone and thymohydroquinone, which shown to posses antimicrobial activities. Also, the antimicrobial activity of coriander seed was studied by Delaquis *et al*. (2002); Singh *et al*. (2002).

Conclusion

It could be concluded that black seed or coriander seed can be used to alleviate the negative effect of heat stress in broiler hematology during the summer season in Egypt. Also, act as an antimicrobial agent.

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References

Abdel-Fattah, S.A., 2006. Physiological and immunological adjustments of dietary ascorbic acid and acetyl salicylic acid in heat stressed Japanese quail. Egyptian Poultry Science 26, 1395 – 1418.

Abou-El-Soud, S.B.A., Ebeid, T.A., Eid, Y.Z., 2006. Physiological and antioxidative effects of dietary acetyl salicylic acid in laying Japanese quail (*Coturnix japonica*) under high ambient temperature. Journal of Poultry Science 43, 255-265.

Abu-Dieyeh, Z.H.M., Abu-Darwish, M.S., 2008. Effect of feeding powdered black cumin seeds on growth performance of 4-8 week old broilers. Journal of Animal and Veterinary Advances 3, 286-290.

Aengwanich, W., Chinrasri O., 2003. Effect of dexamethasone on differential white blood cell counts and heterophil/lymphocyte ratio in Japanese quails (Coturnix *Coturnix japonica*). Songklanakarin Journal of Science and Technology 25, 183-189.

Ahmed, N.A., Maghraby N.A., 1995. Physiological response of broilers to dietary sodium bicarbonate during summer season. Egyptian Journal of Animal Production 31, 271-275.

Ahmed, T., Sarwar, M., Mahr-UN-NISA, Ahsan-UL-HAQ, ZIA-UL-Hasan, 2005. Influence of varying sources of dietary electrolytes on the performance of broilers reared in a high temperature environment. Animal Feed Science 20, 277-298.

Ajakaiye, J.J., Ayo, J.O., Ojo, S.A., 2010. Effects of heat stress on some blood parameters and egg production of Shika Brown Layer chickens transported by road. Biological Research 43, 183- l89.

Al-Obaidi, F.A., Al-Shadeedi, S.M., 2010. Effect of dietary aspirin for reducing a scities and enhancing produetive performance of broilers reared in high density. Al-Qadisiya Journal of Veterinary Medicine Science 9, 20-25.

Altan, O., Altan, A., Çabuk, M., Bayraktar, H., 2000a. Effects of heat stress on some blood parameters in broilers. Turkish Journal of Veterinary and Animal Sciences 24, 145–148.

Altan O., A. Altan, Oguz I., Pabuccuoglu A., Konyalioglu S., 2000b. Effects of heat stress on growth, some blood variables and lipid oxidation in broilers exposed to high Temperature at an early age. British Poultry Science 41, 489-493.

APHA (American Public Health Association), 2005. Standard Methods for the Examination of Water and Wastewater 21th ed. APHA, Inc. Washington, DC.

Aviagen., 2002. Manual of Ross broilers. www.aviagen.com

Delaquis, P.J., Stanich K., Girard B., Mazza, G., 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oil. International Journal of Food Microbiology 74, 101-109.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics 11, l-42.

El-Sheikh, S.E.M., Salama A.A., 2010. Effect of sodium bicarbonate and potassium chloride as water additives on productive performance and egg quality of heat stressed local laying hen in Siwa oasis. Proc. of the 3rd Animal Wealth Research Conf. in the Middle East & North Africa. pp. 108 – 122.

Freeman, B.F., 1969. Physiological responses of the adult fowl to environmental temperature. Poultry Science 22, 140- 145.

Gharib, H.B.A., El- Menawey, M.A., Attalla, A.A., Stino, F.K.R., 2005. Response of commercial layers to housing at different cage densities and heat stress conditions. 1- Physiological indicators and immune response. Egyptian Journal of Animal Production 42, 47-70.

Gharib, H.B.A., Desoky, A.A., El-Menawey, M.A., Ahmed O. Abbas, Hendricks G.L., Mashaly, M.M., 2008. The role of photoperiod and melatonin on alleviation of the negative impact of heat stress on broilers. International Journal of Poultry Science 7, 749-756.

Ghazalah, A.A., EI-Abbady, M.R., Soliman, A.Z.M., Abd-Elsamee, M.O., 1998. Using sodium bicarbonate and ammonium chloride in broiler chick diets to alleviate heat stress in Egypt. Production 35, 199-207.

Gilani, A.H., Jabeen Q., Khan, M.A.U., 2004. A Review of medicinal uses and pharmacological activities of Nigella sativa. Pakistan Journal of Biological Sciences 7, 441-451.

Grey H.G., Paradis T.J., Chang P.W., 1989. Physiological effects of adrenocorticotropic hormone and hydrocortisone in laying hens. Poultry Science 68, 1710-1713.

Guo, F.C., Williams, B.A., Kwakkel, R.P., Li, H.S., Li, X.P., Luo, J.Y., Li, W.K., Verstegen M.W.A., 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poultry Science 83, 175-182.

Hassan, S.M., Mady, M.E., Cartwright A.L., Sabri H.M., Mobarak M.S., 2003. Effect of acetyl salicylic acid in drinking water on reproductive performance of Japanese quail (Coturnix *Coturnix japonica*). Poultry Science 82, 1174-1180.

Hassan M.S.H., El-Sayed, O.A., Namera M.M.M., 2011. Effect of Dietery Sodium Bicarbonate and Potassium Chloride Supplementation on Acid-Base Balance, Plasma Electrolytes and Aldosterone Hormone of Golden Montazah Hens Under Hot Climiate Condition. Egyptian Poultry Sciences 31, 285-303

Keskin, E., Durgan, Z., 1997. Effects of supplemental NaHCO3, KCl, CaCl2, NHCl4 and CaSO4 on acid-base balance, weight gain and feed intake in Japanese quails exposed to constant chronic heat stress. Pakistan Veterinary Journal 17, 60-64.

Konka, Y., Kirkpinar, F., Mert, S., Yurtseven, S., 2009. Effects of dietary ascorbic supplementation on growth performance, carcass, bone quality and blood parame-

ters in broilers during natural summer temperature. Asian Journal of Animal and Veterinary Advances 4, 139-147.

Lee, K.W., Everts, H., Kappert, H.J., Frehner, M., Losa, R., Beynen, A.C., 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. British Poultry Science 44, 450-457.

Mashaly, M.M., Hendricks, G.L., Kalama, M.A., Gehad, A.E., Abbas A.O., Patterson P.H., 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poultry Science 83, 889-894.

Maxwell, M.H., 1993. Avian blood leucocyte responses to stress. World Poultry Science Joural 49, 34-43

Metwally, M.A., 2005. Efficacy different dietary levels of antioxidants vitamin C, E and their combination on the performance of heat stress laying hens. Egyptain Poultry Science 25, 613-636.

Nadia M.A., 2003. A study of some physiological, productive and reproductive parameters of Japanese quail under stress condition" PhD. thesis Department of poultry production. Faculty of Agriculture, El- Fayoum. Cairo University

N.R.C., 1994. Nutrient requirements of poultry". 9th Ed National Academy press, Washington, D.C., USA.

Null, G., 2001. The antioxidant Vitamin –Vitamin C, in www.Vitamincfoundaion.org,

Oruseibio, S.M., Alu, E.U., 2006. Responses of broiler birds to supplementation of Ascorbic acid vitamin C. Nigeria Poultry Science Journal 4, 12-15.

Onu P.N., 2009. Growth Performance, Carcass Characteristics and Economic Benefits of Supplemental Ascorbic On Broilers Starters Exposed To Heat stress. Asian Research Publishing Network (ARPN), 4, 5. www.arpnjournals.com

Osman, A.A., 1996. Effect of heat stress and salts on blood picture of chicken during rearing period. M.Sc. Thesis, Fac. Agric. Cairo Univ., Egypt.

Ramadan D.M. EL-Shoukary, Madeha H.A. Darwish, Mootaz A.M. Abdel-Rahman., 2014. Behavioral, Performance, Carcass Traits and Hormonal Changes of Heat Stressed Broilers Feeding Black and Coriander Seeds. Journal of Advanced Veterinary Research 4, 93-101.

Satterlee D.G., Aquilera-Quintana I.A., Munn B.J., Krautmann B.A., 1989. Vitamin C amelioration of the adrenal stress response in broiler chickens being prepared for slaughter. Comparative Biochemistry and Physiology Part A 94, 569-574.

Singh, G., Kapoor, I.P., Pandey, S.K., Singh, U.K., Singh, R.K., 2002. Studies on essential oils part 10; antimicrobial avtivity of volatile oils of same spices. Phytotherapy Research 16, 680-682.

Sobayo, E.B. Oguntona, O.A. Osinowo, D. Eruvbetine, A.M. Bamgbose, O.A. Adeyemi, A.O. Lomola, E.N. Okeke, Usman J.M., 2008. Effects of Ascorbic acid supplementation on the performance indices of starter pullets in a humid environment. Proceeding of the 33rd Annual conference of Nigerian Society for Animal Production pp. 428-430.

Toyomizu M.T.M., Mujahid A., Akiba Y., 2005. Progressive alteration to core temperature, respiration and blood acid- base balance in broiler chickens exposed to acute heat stress. The Journal of Poultry Science 42, 110-118.

Vijayan, N., Rema, L.P., 1997. Heterophil Lymphocytes ratio a measure of stress in two strains of chicken. Journal of Veterinary Animal Science 28, 37-38.

Zulkifli, I., Che Norma, M.T., Chong, C.H., Loh, T.C., 2000. Heterophil to lymphocyte ratio and tonic immobility reactions to preslaughter handling in broiler chickens treated with ascorbic acid. Poultry Science 79, 402–406.