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Cyclical heat stress and enzyme supplementation affect performance and physiological traits of Japanese quail during the early stage of laying

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This study was carried out to evaluate the effect of heat stress and/or multi-enzyme supplementation on the productivity and physiological parameters of laying Japanese quail from 5 to 11 weeks of age. A total of 132 Japanese quail pullets were distributed in a completely randomized design in the 2 × 2 factorial design, with two ambient temperatures (thermoneutral – TN and heat stress – HS) and two enzyme inclusion levels (0 and 0.01), totaling four treatments with 11 replicates. Productive performance, carcass traits, hemogram and blood biochemical profile were evaluated. The effect of the ambient temperature × enzyme interaction was significant for weight gain, feed conversion rate (P<0.04), red blood cell count and mean corpuscle value. Heat stress reduced (P<0.05) the laying rate and egg mass, the relative weight of feathers, the mean corpuscular hemoglobin and blood glucose level, while it increased (P<0.03) the time to lay the first egg and to reach 25% of egg production. Enzyme supplementation improved (P<0.01) the laying rate, egg mass and blood glucose levels, but

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reduced (P<0.02) packed cell volume, mean corpuscular hemoglobin concentration and blood levels of alanine aminotransferase. Enzyme supplementation improved only the productive performance and blood parameters of quails reared in TN conditions, whereas heat stress impaired productive performance of the birds.

KEY WORDS: enzyme complex / laying performance / poultry feeding

Deleterious effects of high environmental temperature on productivity of broilers, laying hens and quails have been reported [Caurez and Olo 2013, Bonfim *et al*. 2016, Al-Sultan *et al.* 2019, Mehaisen *et al.* 2019]. Moreover, heat stress (HS) produces free radicals by causing oxidative damage to lipids, proteins, nucleic acids and other molecules [Yang *et al.* 2010], while it reduces the size of immune system organs in birds, such as the bursa, spleen and thymus [Fouad *et al*. 2016].

Alternative ways of mitigating the negative effects of HS have been considered, such as the dietary inclusion of vitamin A and E separately [Abd El-Hack *et al.* 2019] or associated to mineral zinc [Caurez and Olo 2013], chromium [Khan *et al.* 2014], polyphenols [Hu *et al*. 2019], propolis [Mehaisen *et al*. 2019], and vitamin C [Shewita *et al.* 2019].

The level of non-starch polysaccharides range from 68 to 94 g/kg in maize, whereas in soybean meal it varies from 170 to 300 g/kg. Starch stores are intracellular and enzymes that break the cell wall may enhance the action of the endogenous amylase [Stefanello *et al.* 2017]. Enzymes result in economic benefits due the improved productive performance and availability of protein/amino acids and metabolizable energy for poultry not exposed to HS, as shown in broilers [Moftakharzadeh *et al.* 2018, Saleh *et al*. 2018] and quails [Kilany and Mahmoud 2014, Rabie and Abo El-Maaty 2015, Grecco *et al*. 2019].

This study was carried out to evaluate the effect of the cyclical heat stress and/or multi-enzyme supplementation on the productivity and physiological parameters of laying Japanese quail from 5 to 11 weeks of age.

Material and methods

The experimental techniques were in accord with the guidelines of the Department Committee of Animal and Poultry Production, according to the official decree of the Ministry of Agriculture in Egypt regarding animal welfare [Decree No. 27 (1967)] that enforces the humane treatment of animals]. A total of 132 female Japanese quail pullets, five weeks of age, were used in a completely randomized design and in the 2 × 2 factorial design, with two ambient temperature and two enzyme inclusion levels (0 and 0.01), totaling four treatments with 11 replicates of three quails allocated to a 25 × 52 × 22 cm cage. Quails started laying between six and seven weeks of age.

Treatments included exposing Japanese quails to two ambient temperatures: thermoneutral or heat stress (HS) for 4 hours daily from 10.00 a.m. to 2.00 p.m. From 5 to 7 weeks of age, the thermoneutral condition was 24°C and the high

Table 1 Composition of experimental diets

Ingredients	Content (kg)
Yellow corn	50.220
Soybean meal (44%CP)	35.770
Dicalcium phosphate	1.160
Limestone	6.140
DL-methionine	0.140
Soybean oil	5.485
Vitamin and Mineral mixture[1]	0.300
NaCl	0.300
Sand	0.485
Total	100.000
Calculated analyses[2]	
Metabolizable energy (Kcal/kg)	2968
Crude protein (%)	20.10
Methionine (%)	0.45
TSAA[3] (%)	0.78
Total lysine (%)	1.09
Calcium (%)	2.90
Available phosphorus (%)	0.35

[1]Vit+Min mixture provides per kilogram of diet: vitamin A, 12,000 IU; Vit. E, 20 IU; Vit. D_3 2,500 IU; Vit. K 1.3 mg; Vit.B_1, 3 mg; Vit. B_2, 5.5 mg; Vit.B_6, 3 mg; Vit. B_{12}, 10 µg. Nicotinic acid, 50 mg Ca pantothenate, 12 mg. d-biotin, 0.50 mg. Folic acid, 1.00 mg. Choline chloride, 600 mg. Trace minerals mg/kg of diet, Zn 60; Mn, 80. Fe, 35; Cu, 8; Se, 0.60.
[2] According to NRC [1994].
[3]Total sulfur amino acids.

ambient temperature was 32°C. The other animals (132 pullets) were kept on their corresponding treatments until 11 weeks of age. From 7 to 11 weeks of age, the thermoneutral condition was 25°C and the high ambient temperature was 33°C. Each group was fed diets (Tab. 1) with or without 0.1% of a commercial multienzyme complex (Avizyme® 1500, Danisco Animal Nutrition), containing 4000 µ/g proteases, 300 µ /g of endo-1,4-β- xylanase, and 400 µ /g of α-amylase.

Birds were individually weighed at 5 and 11 weeks of age to obtain the weight gain. Eggs laid were collected daily and individually weighed accurate to the nearest 0.1 gram. Age at first egg and at 25% of egg production were recorded. The laying rate, egg mass, feed intake and feed conversion rate as feed consumed (g) per produced egg (g) + weight gain (g) were also determined.

At 11 weeks of age, three quails from each treatment were randomly chosen and individually weighed and then slaughtered. The feathers were removed completely by hand picking leaving the skin intact. Thereafter, the abdominal cavity was opened to expose the visceral organs. Then inedible parts (pancreas, spleen, and feathers) and edible parts (liver, heart, and gizzard) were removed and individually weighed accurate to the nearest 0.01 gram. Data were expressed as relative to live body weight.

Three blood samples were collected from each slaughtered bird to heparinized and non-heparinized tubes to obtain the plasma and serum, respectively. Plasma and serum were obtained by centrifugation of blood at 3000 rpm for 20 minutes and were stored at -20°C.

Hemoglobin concentration was determined according to Eilers [1967]. Red blood cells (RBC) were counted on a Bright line hemocytometer using a light microscope at 430X magnification. Blood samples were diluted 200 times with physiological saline solution before counting. White blood cells (WBC) were counted according to Helper (1966). Wintrobe hematocrit tubes were used for the determination of packed cell volume (PCV). Blood samples were centrifuged for 20 min at 4,000 rpm, then PCV values were obtained by reading the packed cell volume on the graduated hematocrit tubes. Obtained results were used to determine mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Plasma and serum evaluated parameters included glucose concentration [Trinder 1969], total protein [Armstrong and Carr 1964], total cholesterol [Watson 1960], alanine aminotransferase [ALT] and aspartate aminotransferase [AST] [Reitman and Frankel 1957], as well as liver glycogen [Allen and Ruff 1981].

Two-way ANOVA was applied to the data and when the F test was significant, means were compared by Tukey's test at P<0.05, using the SISVAR Software [Ferreira 2011].

Results and discussion

The effect of the ambient temperature × enzyme interaction was significant for weight gain and feed conversion rate (P<0.04). Quails reared under HS conditions showed a lower laying rate and egg mass (P<0.04) and took longer to lay the first egg and to reach 25% of egg production (P<0.03). Enzyme supplementation improved (P<0.01) their laying rate and egg mass (Tab. 2).

Weight gain was increased by 12.5% and feed conversion rate was reduced by 18.4% in quails reared under TN conditions and fed diets containing enzymes, with no effect of the enzymes in quails reared under HS conditions. This effect occurred because the used enzymes are capable of increasing the nutrient utilization, as demonstrated by Attia *et al.* [2012] and Barbosa *et al*. [2014], who used a multienzyme product in diets for broilers and observed increased digestibility of dry matter, crude protein, crude fiber, ash and digestible energy. However, this beneficial effect of the enzymes was not able to improve the weight gain and feed conversion rate in quails reared under high temperature conditions.

Heat stress negatively affects animal performance. Layers are susceptible to heat stress due to their high metabolic heat production caused by the increasing egg formation and because of the limited heat dissipation due to the feathers. Another possible reason is that layers subjected to HS may have a decline in ovary weight and in the production of estrogen and progesterone [Attia *et al.* 2016]. Age at first egg is a

Table 2. Productive performance of quails subjected to different ambient temperatures and/or fed diet containing enzymes

Treatments	Weight gain (g)	Feed intake (g/d)	Feed conversion (g/g)	Laying rate (%)	Egg mass (g/d)	OEL (d)	Age at 25% EP (d)
			Effect of ambient temperature (°C)				
TN	85.8	23.2	4.21	30.1[a]	3.54[a]	48.6[b]	59.4[b]
HS	81.0	23.0	4.63	26.3[b]	3.07[b]	50.3[a]	64.5[a]
p value	0.24	0.65	0.01	0.01	0.04	0.03	0.01
			Effect of enzyme inclusion (%)				
0	80.8	22.9	4.65	26.1[b]	3.02[b]	49.9	62.8
0.01	86.1	23.3	4.17	30.3[a]	3.59[a]	49.0	61.1
p value	0.04	0.54	0.01	0.01	0.01	0.16	0.25
			Effect of the ambient temperature × enzyme inclusion				
26 0	81.9[b]	23.2	4.59[a]	27.3	3.13	48.7	59.8
26 0.1	89.8[a]	23.1	3.83[b]	32.8	3.94	48.4	59.0
34 0	79.8[b]	22.5	4.69[a]	24.8	2.91	51.1	65.8
34 0.1	82.1[b]	23.5	4.51[a]	27.7	3.23	49.5	63.2
p value	0.04	0.46	0.01	0.28	0.34	0.29	0.44
SEM	1.4	0.3	0.13	1.4	0.35	0.5	0.8

OEL – onset of egg laying. TN – thermoneutral temperature, HS – heat stress.
Feed conversion ratio= feed intake/ (body weight gain + egg mass).
[ab]Means with different letters in the same column are different at 5% probability.

critical factor for egg-laying performance in poultry, while sexual maturity and egg-laying are dependent on these hormones [Niu *et al*. 2017].

Enzymes are added to animal rations with the objective of increasing diet digestibility, removing antinutritional factors and improving nutrient availability [Zahran *et al*. 2012]. Enzyme supplementation increases egg number and egg mass by 16.1 and 18.8%, respectively, probably as a result of the use of nutrients and energy of the diets. Maize and soybean meal provide targets for NSP enzymes and exogenous protease and amylase may enhance the action of the endogenous enzymes, improving nutrient availability, thus leading to increased egg production. Similar results were found by Perić *et al.* [2011], who demonstrated that laying percentage was increased as a result of the enzyme addition even when low-energy diets were administered to laying hens. Khan *et al*. [2011] tested a multi-enzyme complex in diets with no antibiotics and noted an increase in egg production and egg mass.

There was no effect of the ambient temperature × enzyme interaction on carcass traits ($P>0.05$), whereas heat stress caused a reduction in the relative weight of feathers ($P<0.03$) – Table 3. Variation in the environmental temperature and the enzyme inclusion were not able to affect carcass traits. According to Khalil *et al*. [2012], the increase in environmental temperature during the rearing phase leads to an increase in heat tolerance in Japanese quail and maybe this is the reason for the lack of the HS effect on the carcass and internal organs. Similar results were found by Bonfim *et al.* [2016], who worked with meat-type quails reared under 26 and 32°C and recorded no differences in carcass dressing and percentages of liver and gizzard. However, Attia *et*

Table 3. Carcass traits of quails subjected to different ambient temperatures and/or fed diet containing enzymes

Treatments	Carcass dressing (%)	Liver (%)	Heart (%)	Gizzard (%)	Spleen (%)	Pancreas (%)	Feathers (%)
			Effect of ambient temperature (°C)				
TN	67.7	2.62	0.88	0.60	0.29	0.11	5.83[a]
HS	65.8	2.36	0.87	0.59	0.27	0.07	4.21[b]
p value	0.24	0.20	0.86	0.24	0.22	0.58	0.03
			Effect of enzyme inclusion (%)				
0	67.6	2.62	0.88	0.60	0.32	0.11	5.10
0.01	65.9	2.37	0.88	0.59	0.26	0.07	4.93
p value	0.30	0.21	0.90	0.53	0.19	0.22	0.83
			Effect of the ambient temperature × enzyme inclusion				
26 0	66.6	2.56	0.86	0.59	0.28	0.14	5.96
26 0.1	68.9	2.17	0.89	0.58	0.32	0.07	5.70
34 0	65.3	2.67	0.90	0.61	0.23	0.08	4.25
34 0.1	66.4	2.57	0.86	0.59	0.32	0.06	4.17
p value	0.72	0.46	0.57	0.48	0.34	0.58	0.90
SEM	0.77	0.29	0.15	0.14	0.14	0.11	0.53

TN – thermoneutral temperature, HS – heat stress.
[ab]Means with different letters in the same column are different at 5% probability.

al. [2016] showed a reduction in carcass dressing and the percentages of liver, spleen, ovary and thyroid glands in laying hens reared under HS conditions.

While enzymes improve nutrient use, the nutrient density of the diets was sufficient to maintain the growth of muscles and organs. Kilany and Mahmoud [2014] studied the effect of the enzymes xylanase, proteinase and cellulase in diets for Japanese quails in the raising phase and observed no differences in carcass dressing compared to the control group. The same tend was observed by Grecco *et al.* [2019] using xylanase in diets for meat-type quails from 15 to 35 days old. Similar carcass dressing and percentages of gizzard, liver and heart were reported by Rabie and Abo El-Maaty [2015] when using protease in diets for Japanese quails up to 6 weeks of age compared to the control.

The rate of sensible heat dissipation is determined by the insulation of the feathers, thus feather coverage negatively affects thermoregulation because it impairs the dissipation of internal heat [Cahaner *et al.* 2008]. Feather development is under the control of hormones such as oestrogen and thyroxine. Heat stress may influence such hormonal output and may affect feathering [Leeson and Walsh 2004]. Greater feather percentage was also found by Cooper and Washburn [1998] in broilers reared at 21°C (6.22%) compared to those reared at 32°C (5.03%).

The ambient temperature × enzymes interaction affected RBC counts and mean corpuscular volume (MCV). Lower RBC and higher MCV ($P<0.1$) values were observed in quails reared under heat stress and fed diets with no enzyme supplementation. Heat stress conditions reduced MCH ($P<0.04$), while enzyme inclusion reduced PCV and MCHC ($P<0.01$) – Table 4. The results of our study revealed that the erythrocyte

Table 4. Hemogram profile of quails subjected to different ambient temperatures and/or fed diet containing enzymes

Treatments	RBC (10^6/mL)	Hb (g/dL)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)
Effect of ambient temperature (°C)						
TN	2.54	18.35	36.98	221	94.4[a]	49.7
HS	1.79	16.37	36.97	147	73.4[b]	45.2
p value	0.01	0.05	0.99	0.01	0.01	0.13
Effect of enzyme inclusion (%)						
0	2.16	17.77	39.52[a]	205	84.5	51.8[a]
0.01	2.18	16.96	34.44[b]	163	83.3	43.1[b]
p value	0.89	0.41	0.01	0.01	0.85	0.01
Effect of the ambient temperature × enzyme inclusion						
26 0	2.78[a]	18.74	38.99	142[c]	68.7	48.0
26 0.1	2.30[ab]	17.98	34.96	153[b]	78.2	51.6
34 0	1.53[c]	15.18	40.05	268[a]	100.4	38.2
34 0.1	2.05[b]	17.56	33.92	173[b]	88.4	52.1
p value	0.01	0.12	0.23	0.01	0.11	0.09
SEM	0.09	0.68	0.60	11	4.6	2.08

RBC – red blood count, Hb – hemoglobin, PCV – packed cell volume, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration. TN – thermoneutral temperature, HS – heat stress.
[abc]Means with different letters in the same column are different at 5% probability.

count was influenced by environmental temperature. Enzyme supplementation had no effect on the RBC count in quails reared under TN conditions; however, it improved the RBC count by 34% in birds reared under HS and fed enzyme-supplemented diets.

The reduction in RBC counts may be due to the red cell agglutination caused by HS [Waltz *et al*. 2014]. The significant increase in RBC due to the enzyme supplementation in heat-stressed Japanese quail indicate the beneficial effect of enzymes on the availability of nutrient minerals (iron) for RBC formation.

Birds fed supplemented diets showed similar values of MCV regardless of the environmental temperature, thus indicating the beneficial effect of the enzymes, but when they were fed non-supplemented diets HS caused an 89% increase in MCV values. The obtained values of MCV are in the normal range for female Japanese quails (71 to 197 fl) according to Aina and Ajibade [2014] and Agina *et al*. [2017]. MCV measures the average size of individual red blood cells. A lower RBC count and a higher MCV mean that the volume of the erythrocytes was increased. In red cell agglutination, doublet erythrocytes are counted as one, while larger clumps are not counted as red blood cells at all. This leads to a decrease in RBC count and elevated MCV [Sarma, 1990]. In addition, according to Narkkong *et al.* [2011], the length of the RBC was greater in broilers reared at 38°C compared to those reared at 26°C. The same effect was observed by Maxwell *et al.* [1992] in heat stressed broilers.

A decrease in Hb and MCH is one of the most common findings in livestock during heat stress. Although there was no effect on Hb levels, oxidative damage may have affected Hb molecules, causing their denaturation [Habibu *et al*. 2018]. Decreased MCH values may be related to an increase in MCV in heat-stressed birds, while a higher erythrocyte volume was not followed with an adequate increase of Hb.

There was no effect of the ambient temperature × enzyme interaction on the biochemical parameters; however, heat stress reduced blood glucose level (P<0.01), whereas enzyme supplementation resulted in lower blood levels of glucose (P<0.01) and ALT (P<0.02, Tab. 5).

Table 5. Blood biochemical profile of quails subjected to different ambient temperatures and/or fed diet containing enzymes

Treatments	GLC (mg/dL)	TCHO (mg/dL)	TPT (g/dL)	AST (μ/L)	ALT (μ/L)	AST/ALT	HGLY (mg/100 g)
			Effect of ambient temperature (°C)				
TN	115.4[a]	259	5.21	74.4	30.0	2.79	2.34
HS	95.1[b]	317	4.44	68.1	27.3	2.29	2.24
p value	0.01	0.12	0.23	0.38	0.07	0.11	0.72
			Effect of enzyme inclusion (%)				
0	118.7[a]	322	4.99	75.5	30.5[a]	2.56	2.40
0.01	91.8[b]	254	4.66	67.0	26.9[b]	2.53	2.19
p value	0.01	0.07	0.60	0.24	0.02	0.92	0.45
			Effect of the ambient temperature × enzyme inclusion				
26 0	79.5	324	5.05	65.6	29.6	2.26	2.27
26 0.1	110.7	194	5.37	70.5	30.4	2.32	2.22
34 0	104.2	320	4.28	68.4	24.2	2.85	2.53
34 0.1	126.6	313	4.60	80.5	30.5	2.74	2.16
p value	0.74	0.11	0.99	0.61	0.06	0.77	0.57
SEM	1.6	25	0.37	1.4	0.7	0.30	0.36

GLC – glucose, TCHO – total cholesterol, TPT – total protein, AST – aspartate aminotransferase, ALT – alanine aminotransferase, HGLY – hepatic glycogen. TN – thermoneutral temperature, HS – heat stress.

[ab]Means with different letters in the same column are different at 5% probability.

During HS, adaptations occurred to suppress lipid mobilization [Wheelock *et al.* 2010]. According to Baumgard et al. [2015], metabolism of nonesterified fatty acids results in more metabolic heat than that of carbohydrates. In addition, HS leads to an increased concentration and sensitivity to the plasma insulin as shown by Morera *et al.* [2012] and Fernandez *et al.* [2015] in heat-stressed animals. An increase in the glucose pool coupled with a decrease in blood glucose level suggest a higher rate of glucose leaving the circulating blood pool to be used as fuel by heat-stressed animals [Morera *et al*. 2012].

The used enzymes included amylase and cellulase, in addition to the enzyme protease. Both amylase and cellulase will produce glucose from the breakdown of starch and cellulose, respectively, in the small intestine. However, there was a reduction in blood glucose level as a result of enzyme supplementation. An elevated blood glucose concentration stimulates the release of insulin, which acts on cells of the body to stimulate the uptake, utilization and storage of glucose as glycogen [Holst *et al.* 2016]. So, this is probably the reason for the low blood glucose level in birds fed diets containing enzymes.

Blood glucose, when absorbed into the muscle, is degraded by glycolysis to produce pyruvate that may be used to produce alanine. Alanine is the major gluconeogenic precursor in the hepatocytes. The alanine-pyruvate interconversion is the result of the action of alanine aminotransferase (ALT), an enzyme abundant in the cytoplasm of hepatocytes [Gray *et al.* 2014]. In humans, elevated ALT levels are associated with higher glycemia in individuals with normal glucose regulation [Noordam *et al*. 2017, Huang *et al.* 2019]. Thus, we can speculate that low ALT levels may be associated with low blood glucose levels, corroborating the effect of the enzyme supplementation on blood glucose levels.

Conclusion

Heat stress impairs and enzyme supplementation improves the productive performance and blood parameters in Japanese quails. Enzyme supplementation improves weight gain, feed conversion and blood parameters of birds reared under thermoneutral conditions, whereas it is disadvantageous to birds reared under heat stress conditions.

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