Research Article

Comparative Study of Growth and HSP70 Gene Transcription in Japanese Quails fed Different Levels of Black Soldier Fly, *Hermetia illucens*

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Abstract | Edible insects are considered a promising nutritious, sustainable alternative protein source for feed. The effect of black soldier fly (*Hermetia illucens*) meal on growth performance, heat stress-responses (HS) and heat shock protein (HSP70) gene transcription in gendered Japanese quail was assessed. The quails were fed on three different diets containing 100% soybean meal (diet A), 50% soybean and 50% *H. illucens* meal (diet B) and 100% *H. illucens* meal (diet C). The results revealed that diet B and C significantly increased overall live body weight (LBW), relative carcass weight, small intestine, heart, liver, gender organs, spleen, and bursa of Fabricius of quails when compared to diet A. The LBW, relative carcass weight, and measured organs of the diet A group were significantly impacted by the interaction of dietary treatment and HS, whereas these traits had no or minimal effects on the diet B and diet C groups. The HSP70 gene transcription level was highest in birds exposed to cyclic HS and fed control diet A, and lowest in birds in the diet C groups. Conclusively, 100% replacement of the soybean meal with *H.illucens* meal can enhance the growth response, reduce HSP70 gene transcriptionand mitigate the negative effects of HS on quails exposed to cyclic heat stress.

Keywords | *Hermetia illucens,* quails performance, HSP70, gene transcription, heat stress.

Received | October 03, 2022; **Accepted** | December 20, 2022; **Published** | August 30, 2023
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Citation | El-Sayed MA, Hatab MH, Assi HAEM, Ibrahim NS, Saleh HM, Sayed WAA, Rumpold BA (2023). Comparative study of growth and hsp70 gene transcription in japanese quails fed different levels of black soldier fly, *hermetia illucens*. Adv. Anim. Vet. Sci. 11(9): 1585-1596.
DOI | http://dx.doi.org/10.17582/journal.aavs/2023/11.9.1585.1596
ISSN (Online) | 2307-8316

INTRODUCTION

Edible insects have a major valuable source of protein in livestock feed. They are able to transform low cost material such as organic residues into high quality feed ingredients (Danieli et al., 2016; Makkar et al., 2014; Loponteet al., 2017).In addition, the authorization of a number of insect species as a source of protein for the European poultry industry is anticipated until 2020-2022 (Cutrignelli et al., 2018; Hatab et al., 2020).The use of insects for animal feed as soybean and yellow corn replacement may thus result in regional and sustainable supplies of feed for livestock and might prevent further rain forest deforestation for the cultivation of soybean and yellow corn in the future. In fact, soybean meal is the most popular source of supplemental plant protein for livestock and poultry rations and accounts for nearly 85% of all protein sources used in poultry feeds (Beski et al., 2015; Rada et al., 2017; Hatab et al., 2020).

However, a large percentage of soybean and yellow cornare being imported into most countries, indicating high potential risk and increasing feed cost (Gu et al., 2010). As a result, using edible insects as alternative protein sources ensures continued animal production and has the potential to be a beneficial tool in reducing ration feed costs (Al-Qazzaz et al., 2016; Bovera et al., 2016; Belghit et al., 2018; Schiavone et al., 2016). Black soldier fly, *H. illucens*, and yellow mealworm, *Tenebriomolitor*, are now commercially produced as protein sources in poultry diets (Sayed et al., 2019; Basiouny et al., 2016; Marco et al., 2015; Kawasaki et al., 2019; Hatab et al., 2020).They could play a prominent role as feed because of their high nutrient contents, short life cycles, and can be reared extensively on a large scale using low cost materials as feed substrates (Parker 2005; Sayed et al., 2019). In addition to the high nutritive value of the insect meals, there is unique composition of insect meal protein which provides the bird protection against stresses compared to yellow corn and soybean meal. Due to the insects are poikilothermic organisms (Skendži et al., 2021) and the temperature of their body depends on the temperature of the environment, in addition, they have various heat shock proteins, give them unique feature to adapt, active, live and spread easily in any environmental conditions throughout the year (Wojda and Jakubowicz 2007; Wojda et al., 2009; Guz et al., 2021; Zhao and Jones 2012; Wrońska and Boguś 2020). Many reports conducted by (Wojda and Jakubowicz 2007; Wojda et al., 2009; Wrońska and Boguś 2020) emphasized an important role of heat shock proteins in immune response regulation and improving the resistance of insects to infection under thermal shock conditions. Hence, this research aimed to find out the relation between feeding insect meal and gene transcription of heat shock protein gene. Heat shock proteins (HSPs) are specific proteins that act as molecular biomarkers of different types of stress i.e. food deprivation, bacterial infection and temperature stress (Iwamoto et al., 2008; Cara et al., 2005; Deane and Woo 2005). It has been reported that there is eight HSP induced from chicken (*G. gallus domesticus*) and HSP70 gene transcription is variable in response to different stressors such as food restriction, heavy metals, insecticides, and temperature stress (Yoshimi et al., 2009; Sun et al., 2016; Cedraz et al., 2017). However, some studies reported that the gene may or may not be influenced by the stressors and was present under most conditions in organisms (Morales et al., 2011; Gkouvitsas et al., 2009; Wang et al., 2012).To date, there is no insight on the potential impact of using insect meals as feed ingredient on HSP70 gene transcription of poultry. Hence, it was aimed to study the impact of *H. illucens* meal utilization as partial and whole replacement of yellow corn and soybean meal in Japanese quail diets on growth performance and HSP70 gene transcription.

MATERIALS AND METHODS

Poultry ethics

The scientific committee of the Biological Application Department, Nuclear Research Center, Egyptian Atomic Energy Authority, approved all procedures used in this experiment, number 12PA/23, to the guidelines of the National Institute of Animal Health for animal Care and Use in the experiments.

Insect rearing, harvest and preparation of *H. illucens* meal

The Black Soldier Fly, *H. illucens* was reared and maintained in the insectaria building. The rearing conditions were 65 ± 5% relative humidity and 24 ± 2 °C. The rearing system was started from adult flies that fed on diets consisting of 3:1 of sucrose: yeast hydrolyzate. For drinking, water cups were supplied in the rearing cages. The laid eggs were collected daily. The larvae of *H. illucens* were reared on a semi-artificial diet composed of50.20 % water, 28 % wheat bran, 13 % sugar, 7 % yeast, 0.3 % sodium benzoate, and 1.5 % HCl. After 16 days, the newly formed Pre-Pupae were collected and mashed using Hamiliton blender and then oven-dried for 24 h at 40 °C. The resulting *H. illucens* meal was phyto-sanitary against various pathogens that may infect the insect meal, with minimal adverse effects on the quality of most fresh products (Organization, 1988) through exposingto 80 rad dose rate using a Cobalt 60 gamma cell at a dose rate of 0.03 rad/min. The *H. illucens* meal was kept and stored at room temperature until it used as ingredient to formulate the experimental diets.

Chemical analysis

The chemical compositions of soybean meal and *H. illucens* meal were analyzed in triplicates. The crude (protein, fat and fiber), and ash contents of soybean meal and *H. illucens*meal were analyzed according to Association of Official Analytical Communities AOAC methods (2012). Methionine, lysine and cysteine of soybean meal and *H. illucens* were determined by high-performance liquid chromatography (Beckman Instruments, Inc., Fullerton, CA, USA), where soybean and *H. illucens* protein extracts was applied to a TSK 4000-SW column at a flow rate of 1.0 ml per minute and measured at a detection wavelength of 280nm. The total lipids were determined according to (Folch et al., 1957). Carbohydrate content was determined the by (Albalasmeh et al., 2013) method. Calcium content was determined by Atomic absorption Spectrophotometry (Varian Tectron AA575 series) while, inorganic phosphorus was determined calorimetrically by using Diamond kit produced by Stanbio Company, USA. Oxygen Bomb Calorimeter (Instrumentation India Co.) was used to determine the Gross caloric content.

Birds management

The feeding experiments were conducted on Japanese quails chicks kept at the poultry experimental unit of the Biological Application Department. Three hundred Japanese quail chicks, 10 days-old and weighing 46 g on average were divided randomly into 3 groups (100 chicks in each group, one group for each diet). Each group consisted of 5 pens replicates. Pen dimension was (1.0 m wide X 1.2 m long) and was provided with an automatic drinker and feeder. All groups were farmed in Relative humidity 50 ± 5%, photoperiod (14 L: 10 D) hours and electrically heat-controlled batteries: the first week the temperature was controlled at 35 ˚C, while the rest of experimental period was maintained at 28 ± 2 ˚C. At the last week of experiment , 20 birds (10 ♂ and 10 ♀) in each group, were randomly selected, numbered and exposed for seven consecutive days to 40 ± 2 ˚C for 8 h (from 9:00 to 16:00a.m.) and then to 28 ± 2 ˚C during the remaining experimental period.

Feeding experiments

Three iso-caloric and iso-nitrogenicfeeding treatments were conducted in comparison. In the first treatment, the quails were fed on a control diet A, based on yellow corn and soybean meal. In the second treatment, the quails were fed diet B, where 50% of the soybean meal protein was replaced with *H.illucens* meal. In the third treatment, the quails were fed on diet C, where 100% of the soybean meal protein was substituted with *H. illucens* meal. All Japanese quail diets were formulated according to (NRC,1994) of quails. The three feeding experiments lasted for 6 weeks. Feed and water were provided ad libitum throughout the experimental period. The calculated chemical composition of threeexperimental diets are given in Table 1.

Growth performance and carcass traits

The initial body weight of quail chicks at the beginning of study and the final body weight at the end of the experimental period (6 weeks), were recorded to calculate the body weight gain after the experimental period. At the end of the experimental period of 42 days, 30 quails (6 birds/pen) from each feeding treatment (chosen on the basis of pen average final body weight) (3 female and 3 male) per pen were weighed and slaughtered for carcass analysis. Carcass, liver, heart, proventriculus, gizzard, intestine, spleen, bursa of fabriciusand gender organs for each slaughtered bird were determined and calculated as a relative percentage of LBW. All measurements were performed on the pen basis using a high precision electronic scale. The resulting samples of carcass were stocked at -20ºC for genetic analyses.

Determination of the HSP70 gene transcription

Twelve samples of dram muscle tissues were collected from slaughtered birds at the end of the experimental period (6 weeks) from each treatment were analyzed to determine the transcription of HSP70 gene by Animal Genetic Resources Department, National Gene Bank, Agricultural Research Center, Giza, Egypt, the samples were collected from the Poultry Research Farm of the Biological Application Department, Nuclear Research Center, Egyptian Atomic Energy Authority.

RNA Isolation

The QIA amp RNeasy Mini kit (Qiagen, Germany, GmbH) was used to extract RNA from tissue samples, with 30 mg of tissue sample added to 600µl RLT buffer containing 10µl β-mercaptoethanol per 1ml.Tubes were inserted into adapter sets, which are secured into the clamps of the Qiagen Tissue Lyse, to homogenise samples. High-speed (30 Hz) shaking steps were utilized to produce disruption in 2 minutes. The processes were carried out in accordance with the QI AampRNeasy Mini kit's purification of total RNA from animal tissues methodology after one volume of 70% ethanol was added to the cleared lysate (Qiagen, Germany, GmbH). N.B. To eliminate remaining DNA, DNase digestion was performed on the column.

Oligonucleotide Primers

The primer sequences that were used in the real-time qRT-PCR study are listed in Table (2) along with the primers that were used, which were supplied by Metabion (Germany). A 25µl reaction including 12.5µl of the 2x QuantiTect SYBR Green PCR Master Mix, 0.25µl of Revert Aid Reverse Transcriptase (200 U/L), 0.5µl of each primer at a 20 pmol concentration, 8.25µl of water, and 3µl of RNA template was used to test the primers. The experiment was carried out on a Strata gene MX3005P real-time PCR equipment. The sample was compared to the positive control group using the «ΔΔCt" method described by Yuan et al. (2006), with the following ratio: (2-ct) whereas

ΔΔCt = ΔCt reference – ΔCt target; ΔCt target = Ct control for target gene –Ct treatment for target gene and, ΔCt reference = Ct control for reference gene- Ct treatment for reference gene.

Transcription of HSP70 gene

The SYBR intercalating dye was employed to examine mRNA transcription levels. Hsp70 and the housekeeping gene (*ß. Actin*) were tested using real-time PCR. The transcription levels of *Hsp70* gene and *ß. Actin* as housekeeping gene were assessed in quails exposed to different diet treatments including 50, 100 % replacement of the soybean meal with *H.illucens*meal and control either exposed or non-exposed to high ambient temperatures as shown in (Table 5 and Figures 1 and 2).

Table 1: Composition and calculated analysis of experimental diets of growing Japanese quail

	Experimental diets		
Ingredients [%]	**Diet A (0% *Hermetia illucens* meal)**	**Diet B (50% *Hermetia illucens* meal)**	**Diet C (100 % *Hermetia illucens* meal)**
Yellow corn	42	55.5	66
Soybean meal (44%)	46	19.5	0.0
Hermetia illucens meal	0.0	17.5	30.5
soybean oil	9	4.2	0.0
DL-methionine	0.15	0.15	0.1
Choline chloride	0.05	0.2	0.2
L-Lysine	0.0	0.25	0.5
Dicalcium phosphate	0.8	0.5	0.5
Limestone	1.4	1.6	1.6
Sodium chloride	0.3	0.3	0.3
Vitamin and mineral premix[1]	0.3	0.3	0.3
Calculated values[2] [%]			
Crude protein	24.08	24.07	24.09
Crude fibre	4. 4	4.49	4.78
Lysine	1.34	1.3	1.26
Methionine	0.53	0.59	0.58
Methionine+cysteine	0.6	0.76	0.6
Calcium	0.85	0.86	0.87
available phosphorus	0.31	0.35	0.42
Metabolizable Energy (ME) MJ/kg	13.37	13.57	13.58

1 vitamin-mineral premix provided per kg diet: IU: vit. A 4,000,000, vit. D3 500,000; g: vit. E 16.7, vit. K 0.67, vit. B1 0.67, vit. B2 2, vit. B6 67, vit. B12 0.004, nicotinic acid 16.7, pantothenic acid 6.67, biotin 0.07, folic acid 1.67, choline chloride 400, Zn 23.3, Mn 10, Fe 25, Cu 1.67, I 0.25, Se 0.033, Mg 133.4; 2 calculated according to National Research Council (1994)

Table 2: Target and reference genes, primers sequences, cycling conditions for SYBR green RT-PCR and reference.

Genes	Primers sequences	R.T.	P.D.	Amplification (40 cycles)			Dissociation curve (1 cycle)			Reference
				Secondary denaturation	Annealing (Opticson)	Extension	Secondary denaturation	Annealing	Final denaturation	
Target gene *(Hsp70)*	AACCGCACCACACCCAGCTATG	50°C 30 min.	94°C 15 min.	94°C 15 sec.	65°C 30 sec.	72°C 30 sec.	94°C 1 min.	65°C 30 sec.	94°C 1 min.	Ebrahimi et al., 2015
	CTGGGAGTCGTTGAAGTAAGCG									
Reference gene *(ß. Actin)*	CCACCGCAAATGCTTCTAAAC				51°C 30 sec.			51°C 30 sec.		Yuan et al., 2006
	AAGACTGCTGCTGACACCTTC									

RT: Reverse transcription **P.D:** Primary denaturation ***SYBR green RT-PCR***

Statistical analysis

All variables data of this study were statistically subjected to ANOVA as a completely randomized design using SAS (2012), software version 9.1.3. Differences among means were assessed using Duncan's multiple range tests (Duncan, 1955). The statistical model used in the analysis was as follows: Yijk = μ + Oi + Dj + Ag + ODij + ODAijg + eijk, where Yijk = the observation mean; μ =the overall mean; Oi = the effect of ith dietary treatment; Dj = the effect of jth HS; Ag= the effect of gth gender; ODij = the interaction effect of dietary treatments with HS; ODAijg= the interaction effect of dietary treatments and HS with gender; and eijk = the residual error of the model.

The strata gene MX3005P program analyses the SYBR green RT-PCR findings to determine the amplification curves and CT values in order to evaluate the variance of gene transcription on the RNA of the various samples and the CT of each.

RESULTS

Nutrient Analysis of soybean meal and *H. illucens* meal

The compositions and quality differences of soybean meal and *H. illucens* meal are summarized in Table 3. The metabolizable energy, total protein, total lipid, fiber, calcium, and phosphorus contents of *H. illucens* meal were significantly higher than of soybean meal, while, the carbohydrate content of the *H. illucens* meal was much lower (4.9 %) than of soybean meal (28.51%). The methionine content of *H. illucens* meal was 2 times greater than of soybean meal, while, the lysine content of soybean meal was comparable to *H. illucens* meal. The comparison of the nutrient compositions of soybean meal and *H. illucens* meal suggest that, in view of nutritional value, *H. illucens* meal is better than soybean meal as a feed ingredient. However, the high lipid content of 25.3 % of *H. illucens* meal in comparison to (defatted) soybean meal could be advantageous for either as an energy source or for providing essential fatty acids in feed.

Growth and carcass characteristic of Japanese quail

Effects of Black Soldier Fly, *H. illucens* meal replacement and HS on growth and carcass characteristic of gendered Japanese quail chicks are summarized in Table 4.The overall LBW, the relative weight of carcass, heart, liver, spleen, bursa of Fabricius, small intestine and gender organs of quails were significantly increased (P<0.001) by dietary treatment with *H. illucens* meal and/or HS for diet B and diet C, respectively, as compared with the control diet A. The interaction of the dietary treatment and HS that was applied significantly (P<0.001) affected the LBW, the relative weight of carcass and measured organs of the control group, while the treated groups showed no or the lowest effects on their traits. The statistical analysis of data showed the significant effect of gender on LBW, the relative weight of carcass, heart and liver. The interaction between gender and dietary treatment or HS sinificantly (P<0.001) affect-

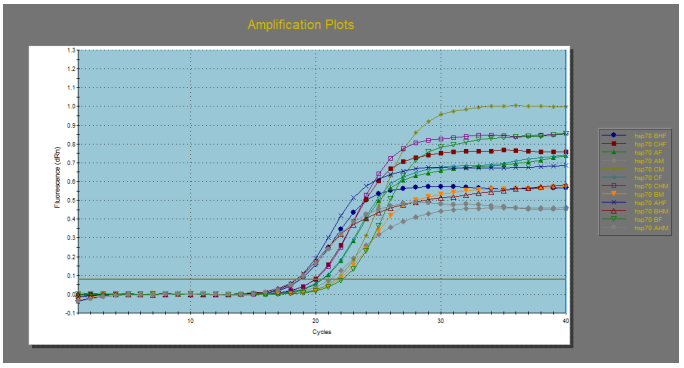

Figure 1: Target HSP70 gene expression in two replacement of soybean meal with *H. Illunces* meal and two treatments groups (exposed and non-exposed to temperatures) of Japanese quail.

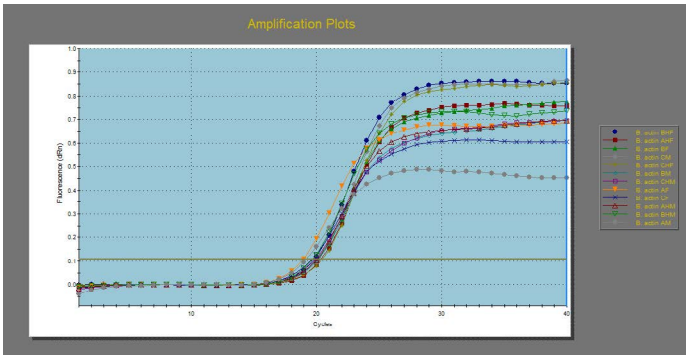

Figure 2: Referance *ß. Actin* gene expression in two replacement of soybean meal with *H. Illunces* meal and two treatments groups (exposed and non-exposed to temperatures) of Japanese quail

Table 3: Average nutrient compositions of soybean meal and *Hermetia illucens* meal in % (based on dry matter).

Nutrient	Soybean meal [%]	*Hermetia illucens* meal [%]
Dry matter	96 ± 0.6	95±0.4
Total protein	43.7 ± 0.9 ^{b}	57.8 ± 0.7 ^{a}
Total lipid	2.3± 0.05 ^{b}	24.9 ± 0.9^{a}
Carbohydrates	30.0 ± 0.1 ^{b}	3.9 ± 0.04 ^{a}
Fiber	8.4 ± 0.05 ^{b}	9.8 ± 0.02 ^{a}
Calcium	1.1 ± 0.02 ^{b}	2.7 ± 0.15 ^{a}
Phosphorus	0.43 ± 0.01 ^{b}	2.89 ± 0.2 ^{a}
Methionine	0.6 ± 0.06 ^{b}	1.19 ± 0.03 ^{a}
Lysine	3.2 ± 0.05 ^{a}	2.2 ± 0.05 ^{b}
Cysteine	0.6 ± 0.1	0.4 ± 0.01
Metabolizable Energy (ME) [kcal/kg]	2229 ^{b}	3302 ^{a}

Means designed with different letters in the same row are significantly different (P≤0.05)

Table 4: Effects of dietary treatments of Black Soldier Fly, Hermetia illucens on Live body weigh (g), relative weight of carcass and selected organs (%) and Small intestine length (cm) of heat stressed Japanese quails (Mean ±SE).

Traits	Gender	Measurements											
		Live body weight	Carcass	Proventriculus	Gizzard	Liver	Heart	Spleen	Bursa	Small intestine	Tests	Ovary	Oviduct
Conditions		Thermo neutral temperature											
Diet A		245.83	69.4	0.34	1.70	1.90	0.67	0.072	0.073	4.19	0.99	0.42	0.15
Diet B		277.0	69.94	0.42	1.76	2.05	0.77	0.09	0.085	4.42	1.9	3.54	1.1
Diet C		280.8	71.61	0.48	1.80	2.48	0.78	0.11	0.111	5.04	3.1	5.5	1.84
SEM		9.96	0.81	0.007	0.04	0.06	0.017	0.006	0.005	0.13	0.3	0.73	0.24
Conditions		Heat stressed											
Diet A		228.15	68.06	0.33	1.71	1. 95	0.62	0.07	0.065	4. 1	0.8	0. 3	0.095
Diet B		273.3	69.02	0.38	1.8	2.17	0.72	0.09	0.08	4.51	1.6	2.87	0.99
Diet C		274.8	71.1	0.42	1. 85	2.32	0.79	0.092	0.104	4.93	2.72	3.9	1.18
SEM		6.45	0.96	0.009	0.05	0.07	0.013	0.038	0.04	0.09	0.13	0.47	0.17
Conditions		Thermo neutral temperature											
Diet A	Male	206.7	71.4	0.42	1.69	1.92	0.68	0.077	0.08	4.01			
Diet B		220.0	71.8	0.43	1.82	2.01	0.76	0.084	0.11	4.45			
Diet C		226.7	72.8	0.44	1.99	2.64	0.81	0.136	0.13	5.52			
SEM		1.63	0.49	0.007	0.073	0.11	0.02	0.009	0.007	0.23			
Conditions		Heat stressed											
Diet A	Male	188.7	69.75	0.4	1.46	1.8	0.65	0.062	0.05	3.84			
Diet B		259.0	71.0	0.4	1.71	1.88	0.73	0.07	0.08	4.24			
Diet C		275.7	71.25	0.41	1.82	2.2	0.78	0.096	0.09	4.81			
SEM		10.2	1.27	0.01	0.09	0.05	0.01	0.076	0.077	0.07			
Conditions		Thermo neutral temperature											
Diet A	Female	265.0	65.4	0.39	1.65	1.88	0.61	0.054	0.06	3.81			
Diet B		318.0	66.5	0.44	1.90	2.19	0.66	0.075	0.069	4.44			
Diet C		328.0	68.8	0.43	1.87	2.32	0.71	0.087	0.097	4.56			
SEM		4.35	1.1	0.01	0.047	0.06	0.019	0.007	0.005	0.11			
Conditions		Heat stressed											
Diet A	Female	247.63	62.7	0.37	1.6	2.38	0.6	0.047	0.058	3.71			
Diet B		271.0	65.0	0.4	1.87	2.46	0.65	0.070	0.091	4.2			
Diet C		290.7	66.1	0.40	1.8	2.73	0.70	0.080	0.12	4.31			
SEM		5.1	0.87	0.01	0.05	0.06	0.025	0.005	0.009	0.15			
Interaction terms		Probability											
Diet		0.00	0.00	0.04	0.00	0.00	0.03	0.007	0.003	0.00	0.00	0.00	0.00
Heat stress		0.003	0.003	0.005	0.00	0.001	0.01	0.006	0.004	0.000	0.00	0.00	0.00
Gender		0.00	0.00	0.87	0.24	0.00	0.00	0.11	0.26	0.99			
Diet * Heat stress		0.00	0.00	0.00	0.00	0.001	0.003	0.002	0.00	0.001	0.01	0.00	0.001
Gender* Heat stress		0.00	0.00	0.00	0.001	0.19	0.002	0.37	0.002	0.00			
Gender* Diet		0.00	0.013	0.06	0.000	0.18	0.00	0.38	0.00	0.05			
Gender* Heat stress * Diet		0.00	0.00	0.00	0.12	0.014	0.37	0.36	0.95	0.00			

Diet A: with 0% *Hermetia illucens* meal **Diet B:** with 50% *Hermetia illucens* meal **Diet C:** with 100% *Hermetia illucens* meal

Table 5: Effect of replacement the soybean meal with *H. illucens* meal on HSP70 Gene Expression in quail

Treatments	Heat stress exposure	ß. actin	*Hsp70*	Expression HSP70 gene
		CT	CT	
Diet A with 0% *H. Illunces meal*	No	19.49	20	
Diet A with 0% *H. Illunces meal*	Yes	20.48	18.29	13.2145
Diet B with 50% *H. Illunces meal*	No	20.41	21.90	
Diet B with 50% *H. Illunces meal*	Yes	20.01	18.43	8.4869
Diet C with 100% *H. Illunces meal*	No	20.17	21.06	
Diet C with 100% *H. Illunces meal*	Yes	20.59	19.86	3.2034

-ed the LBW, the relative weight of carcass and all measured organs except for the relative weight of liver (P < 0.18) and spleen (P < 0.37). The interaction of gender, the dietary treatment and HS sinificantly (P<0.001) affected the LBW, the relative weight of carcass, proventriculus, small intestine and liver. While no significant effects appeared on the relative weight of gizzard (P <0.12), heart (P < 0.37), spleen (P <0.36) and bursa (P < 0.95).

Effect of diets and HS on the gene transcription

The interactions between diets and HS exposure on the gene transcription were observed in this study. The level of HSP70 transcription gene in the control group fed diet A with 0% *H. illucens* meal, was highly significant under high ambient temperature when compared with treated groups fed diet B with 50% *H. illucens* meal or diet C with 100% *H. illucens.* Moreover, the level of HSP70 transcription gene in the muscles of treated group with diet B was greater than the treated group with diet C. Investigating HSP70 gene transcription with HS exposure in control diet A was 13.215. While, the diet B were expressed of 8.487 with the HSP70 gene. Moreover, the gene transcription of the birds fed on diet C were 3.203, respectively as shown in Table (5).

DISCUSSION

Hermetia illucens meal appears to be able to provide protein, methionine, lipids, fiber, calcium, and phosphorus in higher amounts than soybean meal. The obtained results agree with a previous study conducted by Sayed et al., (2019) on the chemical composition of *H. illucens* meal that showed its high nutritive values. Similarly, crude protein content ranged up to 70% of dry matter, lipid content up to 25% of dry matter, higher crude fiber up to 11% in other insect species as reported in other studies (Rumpold and Schlüter 2013; Makkar et al., 2014; Al-Qazzazet al., 2016; Józefiak et al., 2016; Akullo et al., 2018; Schiavone et al., 2017; Spranghers et al., 2017; Hatab et al., 2020). In the present study, the nutrient analysis of *H. illucens*meal and soybean meal clearly confirmed that *H.illucens* meal can be considered a valuable source of energy, protein, methionine and lipids and is thus an excellent alternative protein ingredient for soybean meal in quail diets formulation. Despite the feeding treatments were on iso-caloric and iso-nitrogenic diets and the impact of HS on growth, the aforementioned positive results on LBW and carcass characteristics could be attributed to the nutritional content of *H. illucens* meal as mentioned previously in Table 3 and also may be referred to no anti-nutritive factors present in *H. illucens* meal compared with soybean meal. Soybean meal was characterized by the presence of phytate and anti-nutritive factors such as, trypsin inhibitors and lectins, which severely depressed growth performance in poultry (Gu et al., 2010). The FAO administration in 2014 strongly recommended the inclusion of insect protein in livestock and poultry rations to improve the body weight and carcass characteristics Van Huis et al. (2013). In our results significant differences were observed for quails fed on diet B with 100% *H. illucens* meal and diet A with 50% *H. illucens* meal, respectively, compared with the control diet fed on soybean meal. These findings are coinciding with those observed by other studies (Widjastuti et al., 2014; Bovera et al., 2016; Schiavone et al., 2016; Zotte et al., 2019; Marono et al., 2017; Z Schiavone et al., 2018; Woods et al., 2019; Mbhele et al., 2019). Moreover, the observed interaction between nutrition used and HS that was applied in this study confirmed the high ability of treated diets with *H. illucens* meal on improving the LBW and consequently the carcass characteristics of quails even these birds reared and fed under HS exposure. Furthermore, the significant effect of gender on LBW, the relative weight of carcass, heart and liver along with the interaction present between gender and heat treatment in this study could be explained by the variation between the gender in oxygen consumption, body composition, body temperature and metabolic rate due to the variation in ambient temperature changes (Clarke and Rothery 2007; Hammond et al., 2000; Long et al., 2005; Chatelain et al., 2013). Hence, males had a significantly higher body temperature than females. Nonetheless, females showed higher oxygen consumption than for males (Ward et al., 2002; Moriya et al., 2004; Dart et al., 2002; Green et al., 2001; Chaid, 2020). Unfortunately, the oxygen consumption, body temperature and heart rate were not measured in this study. Thus, in this study, exposing gendered quails fed on *H. illucens* meal to abrupt changes

in ambient temperature "heat stressing", may associated with different gene transcription for HSP70 among dietary treatments. The obtained results in this study indicated that exposing control group fed 0% insect meal to periods of heat chock or hyperthermia induced the body to provide significant transcription of HS shock protein gene in their muscle tissue as protection during stress more than the treated groups that fed 50% or 100% insect meal as a replacement of soybean. However, HSP70 transcription of muscle tissue was not affected by insect meal replacements under thermo-neutral conditions. It is thought that, control group is more susceptible to HS and consequently, it suffers from negative impact on their growth. The adverse effects of exposing to HS on performance, physiological activity, nutrient absorption, digestion, blood circulation, respiration, consumption and utilization of food and sensitivity against several diseases were reviewed previously (Sayed et al., 2019; Hatab et al., 2020). Further studies reported the effect of stress stimuli in triggering the production of heat shock proteins in Japanese quail (Sahin et al., 2009; Kang and Shim 2021).Therefore the heat-stressed quails of control group combat these adverse effects through increasing their HSP70 gene transcription level, this stress protein is important to reduce the negative effects of exposing to HS by repairing the denatured proteins after stress in the process called "thermo tolerance". But, in the case of treated groups with insect meal the transcription of HSP70 gene in their muscle tissue was lower susceptible to HS than the control group which mean that the heat-stressed quails of treated groups in particular group of diet C had low level of muscle HSP70 transcription compared to the control group and consequently, it had better performance under HS exposure. The reduction in the transcription HSP70 gene in treated groups may be attributed to the high nutritive value and unique composition of insect meal protein which provides the bird protection against stresses compared to soybean meal. In this respect, previous finding conducted by (Wu et al., 2018; Fagundes et al., 2020; Bortoluzzi et al., 2018) attributed the positive improvement in growth performance, intestinal development and immune functions of broiler chickens during the hot environment to dietary protein level with well-balanced amino acids. Thus, excellent protein source with high amino acids concentrations in poultry diets under HS must be taken into consideration to compensate the reduction in the protein and amino acids uptake during hot weather conditions (Pearce et al., 2013; Habashy et al., 2017). Moreover, enhancing amino acids-based antioxidant systems via optimizing the dietary supplementation could modulate the oxidative damage induced by HS (Wang et al., 2019). Furthermore, (Sahin et al., 2009; Hidayat and Komarudin, 2020) showed the vital role of dietary amino acids and minerals supplementation in decreasing HS and HSP70 transcription levels in heat-stressed birds. Also, Zhu et al. (2016) reported the role of dietary manganese supplementation in may enhancing the heart's antioxidant ability and inhibiting the transcription of HSP70 in breast muscle. Another interesting observation is the fact that insects themselves respond to abiotic stressors such as elevated temperature, heat shock, viruses, bacteria, and a variety of chemical and physical stresses by a rapid increase in antioxidant activity and production of polypeptides heat shock proteins (Zhao and Jones 2012; Farahani et al., 2020). Hence, the heat shock proteins are abundantly expressed in insects are important modulators involved with insect survival. In the other side of the present study gender of treated birds has been mentioned to examine the pattern of muscle HSP70 gene transcription after heat chock exposure. This aspect is very important as birds have a marked genderual dimorphism, which strongly influences their performance under HS conditions. The results agree with Romani and Russ (2013) who showed greater transcription of Heat shock protein in males vs. female's rats. A number of studies have attributed the induction of heat shock proteins in a variety of tissues, including skeletal muscle to the effect of gender hormones(i.e., estrogen and testosterone) (Paroo et al., 2002; Voss et al., 2003; Al-Madhoun et al., 2007; Nickerson et al., 2006). Moreover, (Long et al., 2005; Chatelain et al., 2013; Clarke and Rothery 2007; Hammond et al., 2000) reportedvariation between the gender in oxygen consumption, body composition, body temperature and metabolic rate due to the variation in ambient temperature changes. Hence, males had a significantly higher body temperature than females. Nonetheless, females showed higher oxygen consumption than for males.

CONCLUSION

The *Hermetia illucens*, could successfully use for poultry feed because it has high nutrient contents and short life cycle as well as may facilitate on large scale with low-cost material. In the view of nutrition, the comparative study between the nutritional compositions of both fly *H. illucens* meal and a soybean meal showed a higher nutritive value of the *H. illucens* better than soybean meal. Thus, when we conducted feeding trials in Japanese quail with *H. illucens* meal up to 100% replacement resulted successfully improvement in the final body weight, and carcass characteristics of Japanese quail chicks compared to the control treatment that fed on *H. illucens*. In the case of impact 50% replacement of the soybean meal with *H. illucens* meal, a slight increase in the final LBW and carcass characterization occurred, while, 100% replacement of the soybean meal with *H. illucens* meal, led to a higher increase in LBW and carcass characterization. Similarly, a higher change in HSP70 gene transcription and a noticeable change in HSP70 gene transcription of Japanese quail

meat at 50% and 100% soybean meal replacement, respectively were identified in comparison to the quail's original genome and the control treatment. The transcription gene in control was higher than in the case of the replacement soybean with 50 and 100 % of *H. Illunces* meal. Also, the interactions between HS and diet were observed in our study. Finally, the added insect of *H. illucens* significantly increased food intake and reduced HSP70 gene transcription in muscle tissue of heat stresed quail compared with the control-treated group. Therefore when quails fed on *H. illucens* meal as a replacement percentage of soybeans in the diet reach 100% decrease the level of Hsp70 and acquired these birds' levels and low in the transcription of this gene that the result from exposure to HS, and as a result, reduce the harmful effects of HS. It is possible to conclude that *H. illucens* meal can be employed as a dietary strategy for heat stress mitigation in Japanese quail using the HSP70 gene transcription indicator.

ABBREVIATIONS

H .illucens: Hermetia illucens,
HS: heat stress-responses,
HSP: heat shock protein,
diet A: diets containing 100% soybean meal,
diet B: 50% soybean and 50% Hermetia illucens meal,
diet C: 100% Hermetia illucens meal,
LBW: live body weight.

ACKNOWLEDGEMENTS

The authors also would like to express sincere thanks and deep appreciation to the Biological Application Department, Nuclear Research Center, Egyptian Atomic Energy Authority, for their cooperation and the National Gene Bank, Animal Genetic Resources Dept., Agric. Res. Center, Giza, Egypt.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

NOVELTY STATEMENT

The main objective of this paper is to examine the effect of black soldier fly (*Hermetia illucens*) meal as a partial and complete replacement for soybean meal protein in Japanese quail diets on growth performance, heat stress-responses and heat shock protein (HSP70) gene transcription in gendered Japanese quail. This insect has high-quality protein, amino acids, peptides, fats, chitin, minerals, and vitamins-rich biomass, as well as having the ideal antioxidant and immune-boosting properties for processing animal feed. It has beneficial effect of enhancing growth performance and reducing feed costs. In addition, it has various heat shock proteins, that gives them unique feature to active and adapt easily. Numerous studies shown that insects have the greatest tolerance to high temperatures, highly adaptable and able to survive in different climatic conditions due to their ability to stimulate defense mechanisms against thermal stress which due to heat shock proteins (HSPs). They are involved in protecting proteins, expressing heat shock genes and increasing the enzymatic antioxidant activity in response to heat stress and can also enhance the physiological and immune responses. No research has been conducted to determine the relationship between feeding *H. illucens* meal and the transcription of the HSP70 gene in Japanese quails in response to heat stress. The results revealed that, incorporating *H. illucens* meal into the diet of heat-stressed Japanese quails can effectively improve growth performance and alleviate the negative impacts of heat stress as indicated by the reduction in HSP70 gene transcription. The study introduced effective strategy for the poultry industry, as they provide a potential solution to improve productivity in birds raised in hot environments.

AUTHORS CONTRIBUTION

Conceptualization, N.S.I., M.H.H. and W.A.A.S.; Data curation, M.A El., N.S.I. and M.H.H.; Formal analysis, N.S.I.; Investigation, W.A.A.S., N.S.I. and M.H.H.; Methodology, N.S.I., M.H.H., H. AEM. A., M.A El., and W.A.A.S.; Resources, W.A.A.S., N.S.I., M. A. El and M.H.H.; Software, N.S.I.; Validation, N.S.I. and M.H.H.; Visualization, W.A.A.S., N.S.I., M.H.H., H. M. S. and B.A.R.; Writingoriginal draft, N.S.I., W.A.A.S., and M.A El.; Writing review & editing, N.S.I., W.A.A.S. and M.A. El.

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