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Comparative Study Of Growth And HSP70 Gene Expression In Japanese Quails Fed Different Levels Of Black Solder 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 solder fly (BSF), *Hermetia illucens* meal on growth performance, heat stress-responses and heat shock protein (HSP70) gene expression in sexed Japanese quail were assessed. The quails were fed on three different diets containing 100% soybean meal (diet A), 50% soybean and 50% BSF meal (diet B) and 100% BSF meal (diet C). Results revealed that overall live body weight, the relative weight of carcass, heart, liver, spleen, bursa of Fabricius, small intestine and sex organs of Japanese quail were significantly increased for diet B and C as compared to diet. The interaction of dietary treatment and heat stress had a significant effect on the diet A group's live body weight, the relative weight of carcass, and measured organs, whereas the diet B and diet C groups had no or the least effect on their traits. Investigating HSP70 gene expression with heat stress exposure in control diet A was 13.215. While, the diet B was expressed of 8.487 with the HSP70 gene. Moreover, the gene expression of the birds fed on diet C were 3.203, respectively. Our findings point to a beneficial role for BSF meal as a quail feed in improving growth performance and combating the negative effects of heat stress exposure on bird growth.

Introduction

Edible insects have a major potential as an alternative source of protein in livestock feed. They are able to transform low cost material such as organic residues into high-quality ingredients for feed products with limited pressure on land, water and energy resources (Makkar et al. 2014; Danieli et al. 2016; Loponte et al. 2017). In addition, the authorization of a number of insect species as a protein source for the European poultry industry is anticipated until 2020–2022 Cutrignelli et al. (2018).The use of edible insects for animal feed as soybean replacement may thus result in regional and sustainable supplies of feed for livestock and might prevent further rain forest deforestation for the cultivation of soy in the future. Admittedly, 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). However, a large percentage of soybean is being imported into most countries, indicating high potential risk and increasing feed cost Gu et al. (2010). Therefore, the use of edible insects as alternative protein sources guarantees the continuous animal production and has potential to be a valuable tool in lowering ration feed costs (Al-Qazzaz et al. 2016; Bovera et al. 2016; Schiavone et al. 2016; Belghit et al. 2018). Nowadays, black soldier fly, *Hermetia illucens*, and the yellow mealworm, Tenebrio molitor, are commercially produced as a source of protein in poultry diets (Marco et al. 2015; Kawasaki et al.2019). Additionally, more recent researches have evaluated the potential value of insect meal as poultry feed derived from another insect species. New avenues were opened for the utilization of the common cutter biller, Spodoptera littoralis larvae and/or the peach fruit fly, Bactrocera zonata as sources of protein as feed (Basiouny et al. 2016; Sayedet al. 2019; Hatab et al. 2020). B. zonata could play an prominentrole as feed because it has short life cycles and can be reared on a large scale using low cost material as feed substartes like other fruit fly species Parker (2005). Also, it has high nutrient contents Sayedet al. (2019). While, many studies supported the use of edible insects, a number of challenges remain. Hence, this study aimed to explore the relation of feeding insect meal and gene expression. Heat Shock Proteins (HSPs) are specific proteins that act as molecular biomarkers of various types of stress such as food deprivation, bacterial infection and temperature stress (Cara et al. 2005; Iwamoto et al. 2008; Deane and Woo 2005). Of all HSPs, Heat-shock protein 70 (HSP70) is taking part in cytokine secretion and protein synthesis, folding, transporting and degradation that plays a key role in homeostasis and immune responses (Tsan and Gao 2009;Stankowski et al. 2011). It has been suggested that HSP70 gene expression is variable in response to different stressors such as food restriction, insecticides, heavy metals 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 (Gkouvitsas et al. 2009; Moraleset al. 2011; Wang et al. 2012).To date, there is no insight on the potential impact of using insect meals as feed ingredient on HSP70 gene sequencing and/or expression of poultry. Therefore, in this study the HSP70 sequence of Japanese quails was examined that fed on two levels of *Hermetia illucens* meal compared with those fed on only soybean meal. Hence, it was aimed to study the impact of *Hermetia illucens* meal utilization as partial and whole replacement of soybean meal in Japanese quail diets on growth performance and HSP70 gene sequencing.

Materials And Methods

Poultry ethics

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

Insect Rearing, Harvest And Preparation Of Hermetia Illucens Meal

The Black Solder Fly, *Hermetia illucens* was reared and maintained in the insectaria building. The rearing conditions were 24 ± 2°C, 65 ± 5% relative humidity. 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 *Hermetia illucens* were reared on a semi-artificial diet that was developed by Tanaka et al. (1969) consisting of 28% wheat bran, 7% yeast, 13% sugar, 0.3% sodium benzoate, 1.5% HCl and 50.20% water. After 16 days, the newly formed Pre-Pupae were collected, ground using a blender (3.8 liter) (Hamiliton Beach blender model HBF900S, VA, USA) and then oven-dried at 40°C for 24 h. The resulting BSF meal was irradiated with an 80 krad dose rate using a Cobalt 60 gamma cell at a dose rate of 0.03 krad /min. Irradiation treatment is an effective phytosanitary treatment against various pathogens or unfavorable pests that may infect the insect meal, with minimal adverse effects on the quality of most fresh products (Organization, 1988). The *H. illucens* meal was kept and stored at room temperature used as ingredient in feeding experiments to formulate diets along with other ingredients.

Chemical analysis

The chemical compositions of soybean meal and *Hermetia illucens* meal were analyzed in triplicates. The crude protein, crude fat, crude fiber and ash contents of soybean meal and B. zonata meal were analyzed according to Association of Official Analytical Chemists (AOAC) methods (2012). The contents of the amino acids methionine, lysine and cysteine of soybean meal and B. zonata were analyzed by high-performance liquid chromatography (Beckman Instruments, Inc., Fullerton, CA, USA), where soybean and *Hermetia illucens* protein extracts was applied to a TSK 4000-SW column at a flow rate of 1.0 ml min-1 and measured at a detection wavelength of 280nm (0.02 AUFS). Proteins were eluted isocratically by 0.01 M potassium phosphate buffer (pH 7.4). The total lipids were extracted and purified according to Folch et al. (1957). Carbohydrate content was estimated according to the method by Albalasmeh et al. (2013). Gross caloric content was determined by using an Oxygen Bomb Calorimeter (Instrumentation India Co.). Calcium content was determined by Atomic absorption Spectrophotometry (Varian Tectron AA575 series). Inorganic phosphorus was determined colorimetrically using commercial Diamond kit produced by Stanbio Company, USA using a computerized spectrophotometer model Milton Roy 1201.

Birds and husbandry

The feeding experiments were conducted on Japanese quails chicks maintained at the poultry experimental farm of the Biological Application Department. A total of 300 Japanese quail chicks, 10 days-old and weighing 46 g on average were randomly allotted to three groups (100 chicks in each group, one group for each diet). Each group consisted of five pens as replicates. Each pen was 1.0 m wide X 1.2 m long and was equipped with a feeder and an automatic drinker. All groups were farmed in electrically heat- controlled batteries: the first week the temperature was controlled at 35 ˚C, while 28 ± 2 ˚C was maintained after that until the end of the experiment. Relative humidity was 50 ± 5% and photoperiod was 14 L: 10 D hours. 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 16:00 a.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 based on yellow corn and soybean meal. In the second treatment, the quails were fed diet A, where 50% of the soybean meal protein was replaced with Hermetia illucens meal. In the third treatment, the quails were fed on diet B, where 100% of the soybean meal protein was substituted with Hermetia illucens meal. All diets were formulated to meet the nutrient requirements of Japanese quail according to NRC, (1994). The three feeding experiments lasted for 6 weeks. Feed and water were provided ad libitum throughout the experimental period. The calculated chemical composition of the control and experimental diets are given in Table 1.

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
Bactrocera zonata 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	1.8
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	24.01	24.09
Crude fibre	4.14	4.49	4.78
Lysine	1.45	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)

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 fabricius and sex organs for each slaughtered bird were determined and calculated as a relative percentage of live body weight. 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.

Genetic analysis

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 and gene of HSP70 expression 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

RNA extraction from tissue samples was applied using QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH) when 30 mg of the tissue sample was added to 600 µl RLT buffer containing 10 µl β-mercaptoethanol per 1 ml. For homogenization of samples, tubes were placed into the adaptor sets, which are fixed into the clamps of the Qiagen Tissue Lyse. Disruption was performed in 2 minutes high-speed (30 Hz) shaking step. One volume of 70% ethanol was added to the cleared lysate, and the steps were completed according to the purification of total RNA from animal tissues protocol of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH). N.B. On column DNase digestion was done to remove residual DNA.

Oligonucleotide Primers

Primers used were supplied from Metabion (Germany) are listed and PCR conditions as shown in table (2) lists the primer sequences that were used in the real-time qRT-PCR analysis.

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)			R
				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.	El et 2(
	CTGGGAGTCGTTGAAGTAAGCG									
Reference gene *(ß. Actin)*	CCACCGCAAATGCTTCTAAAC				51˚C 30 sec.			51˚C 30 sec.		Yu al
	AAGACTGCTGCTGACACCTTC									

RT: Reverse transcription **P.D**: Primary denaturation

SYBR green RT-PCR

Primers were utilized in a 25µl reaction containing 12.5 µl of the2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 0.25 µl of RevertAid Reverse Transcriptase (200 U/µL) (Thermo Fisher), 0.5 µl of each primer 20 pmol concentration, 8.25 µl of water, and 3 µl of RNA template. The reaction was performed in a Strata gene MX3005P real-time PCR machine. The sample was compared with that of the positive control group according to the "ΔΔCt" method stated by Yuan et al. (2006) using the following ratio: (2-ΔΔct) whereas ΔΔCt = ΔCt reference – ΔCt target; ΔCt target = Ct control –Ct treatment and, ΔCt reference = Ct control- Ct treatment

Expression HSP70 gene

SYBR intercalating dye was used to analyze mRNA level expression. Real-time PCR was carried out for Hsp70 and housekeeping gene (*ß. Actin*).Two genes of *ß. Actin* and HSP70 gene was expressed with treatments 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 Figs. 1 and 2).

Statistical analysis

Data of this study for all variables 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 heat stress; Ag = the effect of gth sex; ODij = the interaction effect of dietary treatments with heat stress; ODAijg = the interaction effect of dietary treatments and heat stress with sex; and eijk = the residual error of the model.

For the genetic study, a one-way analysis of variance was done using SAS (2012) software version 9.1.3, following the General Linear Model procedure with dietary treatment as fixed effects. Mean values assessed for significance using (Duncan, 1955) multiple range tests. Finally, analysis of the SYBR green RT-PCR results amplification curves and CT values were determined by the strata gene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each.

Results And Discussion

Nutrient Analysis of soybean meal and Hermetia illucens meal

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

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	97 ± 0.23	96 ± 0.35
Total protein	44.0 ± 1.5 ^{b}	58.1 ± 0.4 ^{a}
Total lipid	1.9 ± 0.02 ^{b}	25.3 ± 0.08 ^{a}
Carbohydrates	28.51 ± 0.83 ^{b}	4.9 ± 0.02 ^{a}
Fiber	7.3 ± 0.52 ^{b}	10.9 ± 0.04 ^{a}
Calcium	1.03 ± 0.02 ^{b}	4.79 ± 0.11 ^{a}
Phosphorus	0.41 ± 0.01 ^{b}	8.80 ± 0.14 ^{a}
Methionine	0.6 ± 0.69 ^{b}	1.14 ± 0.04 ^{a}
Lysine	2.99 ± 0.03 ^{a}	2.32 ± 0.05 ^{b}
Cysteine	0.66 ± 0.075	0.5 ± 0.004
Metabolizable Energy (ME) [kcal/kg]	2230 ^{b}	3300 ^{a}
Means designated with different letters in the same row are significantly different ($p \leq 0.05$).		

Growth and carcass characteristic of Japanese quail

Effects of Black Solder Fly, *Hermetia illucens* meal replacement and heat stress on growth and carcass characteristic of sexed Japanese quail chicks are summarized in Table 4. The overall live body weight, the relative weight of carcass, heart, liver, spleen, bursa of Fabricius, small intestine and sex organs of Japanese quail were significantly increased ($P < 0.001$) by dietary treatment with *Hermetia illucens* meal and/ or heat stress for diet B and diet C, respectively, as compared with the control diet A. The interaction of the dietary treatment and heat stress that was applied significantly ($P < 0.001$) affected the live body weight, 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 sex on live body weight, the relative weight of carcass, heart and liver. The interaction between sex and dietary treatment or heat stress sinificantly ($P < 0.001$) affected the live body weight, 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 sex, the dietary treatment and heat stress sinificantly ($P < 0.001$) affected the live body weight, 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 ($P < 0.95$).

Table 4
Effects of dietary treatments of Black Solder Fly, Hermetia illucens on Live body weight[g], relative weight of carcass and selected organs [%]and Small intestin stressed Japanese quails (Mean ± SE).

Experimental treatments	Conditions		Measurements									
			Live body weight	Carcass	Proventriculus	Gizzard	Liver	Heart	Spleen	Bursa	Small intestine	Tests
Diet A with 0% *Hermetia illucens* meal	**Normal heat temperature**		245.83± 8.50	69.4± 1.4	0.34± 0.01	1.70± 0.11	1.90± 0.03	0.67± 0.009	0.072± 0.007	0.073± 0.003	4.19± 0.13	0.99± 0.02
Diet B with 50% *Hermetia illucens* meal			277.0± 9.44	69.94± 0.2	0.42± 0.02	1.76± 0.04	2.05± 0.06	0.77± 0.03	0.09± 0.005	0.085± 0.001	4.42± 0.1	1.9± 0.04
Diet C with 100% *Hermetia illucens* meal			280.8± 9.30	71.61± 1.2	0.48± 0.01	1.80± 0.04	2.48± 0.07	0.78± 0.03	0.11± 0.001	0.111± 0.001	5.04± 0.03	3.1 ± 0.11
Diet A with 0% *Hermetia illucens* meal	**Heat stressed**		228.15± 8.80	68.06± 1.08	0.33± 0.01	1.71± 0.06	1. 95± 0.05	0.62± 0.022	0.07± 0.120	0.065± 0.003	4. 1± 0.08	0.8± 0.07
Diet B with 50% *Hermetia illucens* meal			273.3± 5.45	69.02± 1.36	0.38± 0.01	1.8± 0.06	2.17± 0.13	0.72± 0.027	0.09± 0.009	0.08± 0.11	4.51± 0.059	1.6 ± 0.31
Diet C with 100% *Hermetia illucens* meal			274.8± 7.34	71.1± 1.45	0.42± 0.02	1. 85± 0.04	2.32± 0.18	0.79± 0.015	0.092± 0.003	0.104± 0.009	4.93± 0.26	2.72 ± 0.07
		Sex										
Diet A with 0% *Hermetia illucens* meal	**Normal heat temperature**	**Male**	206.7± 0.88	71.4± 0.32	0.42± 0.01	1.69± 0.04	1.92± 0.03	0.68± 0.01	0.077± 0.001	0.08± 0.001	4.01± 0.06	
Diet B with 50% *Hermetia illucens* meal			220.0± 2.08	71.8± 0.34	0.43± 0.01	1.82± 0.03	2.01± 0.02	0.76± 0.001	0.084± 0.002	0.11± 0.006	4.45± 0.06	
Diet C with 100% *Hermetia illucens* meal			226.7± 4.42	72.8± 0.32	0.44± 0.01	1.99± 0.03	2.64± 0.02	0.81± 0.02	0.136± 0.008	0.13± 0.002	5.52± 0.17	
Diet A with 0% *Hermetia illucens* meal	**Heat stressed**	**Male**	188.7± 0.88	69.75± 0.96	0.4± 0.015	1.46± 0.026	1.8± 0.03	0.65± 0.01	0.062± 0.24	0.05± 0.004	3.84± 0.09	
Diet B with 50% *Hermetia illucens* meal			259.0± 4.9	71.0± 0.68	0.4± 0.02	1.71± 0.03	1.88± 0.03	0.73± 0.009	0.07± 0.002	0.08± 0.2	4.24± 0.1	
Diet C with 100% *Hermetia illucens* meal			275.7± 3.48	71.25± 1.2	0.41± 0.00	1.82± 0.036	2.2± 0.02	0.78± 0.01	0.096± 0.001	0.09± 0.004	4.81± 0.01	
Diet A with 0% *Hermetia illucens* meal	**Normal heat temperature**	**Female**	265.0± 2.89	65.4± 0.64	0.39± 0.012	1.65± 0.007	1.88± 0.03	0.61± 0.02	0.054± 0.001	0.06± 0.002	3.81± 0.05	
Diet B with 50% *Hermetia illucens* meal			318.0± 4.17	66.5± 1.53	0.44± 0.029	1.90± 0.053	2.19± 0.04	0.66± 0.01	0.075± 0.01	0.069± 0.001	4.44± 0.05	
Diet C with 100% *Hermetia illucens* meal			328.0± 2.31	68.8± 0.25	0.43± 0.006	1.87± 0.056	2.32± 0.05	0.71± 0.036	0.087± 0.010	0.097± 0.003	4.56± 0.057	
Diet A with 0% *Hermetia illucens* meal	**Heat stressed**	**Female**	247.63± 4.84	62.7± 0.64	0.37± 0.03	1.6± 0.037	2.38± 0.06	0.6± 0.021	0.047± 0.003	0.058± 0.004	3.71± 0.16	

Experimental treatments	Conditions	Measurements								
		Live body weight	Carcass	Proventriculus	Gizzard	Liver	Heart	Spleen	Bursa	Small intestine	Tests
Diet B with 50% *Hermetia illucens* meal		271.0± 6.67	65.0± 1.52	0.4± 0.01	1.87± 0.063	2.46± 0.07	0.65± 0.024	0.070± 0.005	0.091± 0.004	4.2± 0.06	
Diet C with 100% *Hermetia illucens* meal		290.7± 2.34	66.1± 0.15	0.40± 0.02	1.8± 0.01	2.73± 0.07	0.70± 0.03	0.080± 0.004	0.12± 0.01	4.31± 0.31	
Interaction terms		**Probability**									
Nutrition		0.00	0.00	0.04	0.00	0.00	0.03	0.007	0.003	0.00	0.00
Heat stress		0.003	0.003	0.005	0.00	0.001	0.01	0.006	0.004	0.0003	0.00
Heat stress * Nutrition		0.00	0.00	0.00	0.00	0.001	0.003	0.002	0.00	0.001	0.01
Sex		0.00	0.00	0.87	0.24	0.00	0.00	0.11	0.26	0.99	
Sex* Heat stress		0.00	0.00	0.00	0.001	0.19	0.002	0.37	0.0023	0.00	
Sex* Nutrition		0.00	0.013	0.067	0.0001	0.18	0.00	0.38	0.00	0.017	
Sex* Heat stress * Nutrition		0.00	0.00	0.00	0.12	0.014	0.37	0.36	0.95	0.0001	

Effect of diets and heat stress on the gene expression

The interactions between diets and heat stress exposure were observed in this study. The level of HSP70 expression 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 expression gene in the muscles of treated group with diet B was greater than the treated group with diet C. Investigating HSP70 gene expression with heat stress exposure in control diet A was 13.215. While, the diet B were expressed of 8.487 with the HSP70 gene. Moreover, the gene expression of the birds fed on diet C were 3.203, respectively as shown in table (5).

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

Treatments	Heat stress exposure	*ß. actin* CT	*Hsp70* CT	Expression HSP70 gene
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

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 Hermetia 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 (Makkar et al. 2014; Al-Qazzaz et al. 2016; Hatab et al. 2020; Rumpold and Schlüter 2013; Józefiak et al. 2016; Akullo et al. 2018; Schiavone et al. 2017; Spranghers et al. 2017). In short, the nutrient analysis of Hermetia illucens meal and soybean meal clearly confirmed that Hermetia 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 in the present study. Despite the feeding treatments were on iso-caloric and iso-nitrogenic diets and the impact of heat stress on growth, the aforementioned positive results on live body weight and carcass characteristics could be attributed to the nutritional content of Hermetia illucens meal as mentioned previously in Table 3 and also may be referred to no anti-nutritive factors present in Hermetia 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% Hermetia illucens meal and diet A with 50% Hermetia illucens meal, respectively, compared with the control diet fed on soybean meal. These findings are coinciding with those observed by other studies (Bovera et al. 2016; Schiavone et al. 2016; Widjastuti et al. 2014;Zotte et al.

2019; Maronoet al. 2017; Z Schiavone et al. 2018; Mbhele et al. 2019; Woods et al. 2019). Moreover, the observed interaction between nutrition used and heat stress that was applied in this study confirmed the high ability of treated diets with Hermetia illucens meal on improving the live body weight and consequently the carcass characteristics of quails even these birds reared and fed under heat stress exposure. Furthermore, the significant effect of sex on live body weight, the relative weight of carcass, heart and liver along with the interaction present between sex and heat treatment in this study could be explained by the variation between the sexes 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. Unfortunately, the oxygen consumption, heart rate and body temperature were not measured in this study. Thus, in this study, exposing sexed quails fed on Hermetia illucens meal to abrupt changes in ambient temperature "heat stressing", was associated with different gene expression 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 expression of Heat Stress 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 expression of muscle tissue was not affected by insect meal replacements under thermo-neutral conditions. It is thought that, control group is more susceptible to heat stress and consequently, it suffers from negative impact on their growth. The adverse effects of exposing to heat stress on performance, physiological activity, nutrient absorption, digestion, blood circulation, respiration ,consumption and utilization of food and sensitivity against several diseases etc. are well reviewed by (Sayed et al. 2019; Hatab et al. 2020). In further study conducted by (Sahin et al. 2009; Kang and Shim 2021) reported the role of stress factors in inducing the expression of Heat shock proteins in the Japanese quail. Therefore the heat-stressed quails of control group combat these adverse effects through increasing their expression of Heat Stress shock protein gene, this stress protein is critically important to reduce the negative effects of exposing to heat stress by repairing the denatured proteins after stress in the process called “thermotolerance”.But, in the case of treated groups with insect meal the expression of HSP70 gene in their muscle tissue was lower susceptible to heat stress 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 expression compared to the control group and consequently, it had better performance under heat stress exposure. The reduction in the expression 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 findings conducted by (Bortoluzzi et al. 2018; Wu et al. 2018;Fagundes et al. 2020) reported a positive response to dietary protein level with well-balanced amino acids during the hot environment to improve growth performance, intestinal development and immune functions of broiler chickens. Thus, excellent protein source with high amino acids concentrations in poultry diets under heat stress must be taken into consideration to compensate the reduction in the protein and amino acids uptake during hot weather conditions (Habashy et al. 2017; Pearce et al. 2013). Moreover, enhancing amino acids-based antioxidant systems via optimizing the dietary supplementation could modulate the oxidative damage induced by heat stress Wang et al. (2019). Furthermore, (Sahin et al. 2009; Hidayat and Komarudin 2020) showed the vital role nutritional approaches such as dietary vitamin C or E, plant bioactives, amino acids, probiotics, prebiotics, synbiotics, mannan oligossaccharides and minerals (selenium, zinc, manganese, chromium). Supplementation in decreasing heat stress and HSP70 expression 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 expression of HSP70 in breast muscle. Another interesting observation is the fact that in this study, the rich content of Black Solder Fly, Hermetia illucens in heat shock protein gene, give the insect unique feature to live in any environmental conditions either hot or cold (Senlin et al. 2017; Cardinaletti et al. 2019). In the other side of the present study gender of treated birds has been mentioned to examine the pattern of muscle HSP70 gene expression after heat chock exposure. This aspect is very important as birds have a marked sexual dimorphism, which strongly influences their performance under heat stress conditions. The results agree with Romani and Russ (2013) who showed greater expression of Heat shock protein in males vs. females 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 sex hormones (i.e., testosterone in males and estrogen in females) (Al-Madhoun et al. 2007; Nickerson et al.2006; Paroo et al. 2002; Voss et al. 2003). Moreover, (Clarke and Rothery 2007; Hammond et al. 2000;Long et al. 2005; Chatelain et al.2013) reported variation between the sexes 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 BSF *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 BSF. In the case of impact 50% replacement of the SP with *H. illucens* meal, a slight increase in the final live body weight and carcass characterization occurred, while, 100% replacement of the BSF with *H. illucens* meal, led to a higher increase in live body weight and carcass characterization. Similarly, a higher change in HSP70 gene expression and a noticeable change in HSP70 gene expression of Japanese quail meat at 50% BSF replacement and 100% BSF replacement, respectively were identified as compared to the control treatment as well as the original genome of quail. The expression 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 heat stress and diet were observed in our study. Finally, the added insect of *H. illucens* significantly increased food intake and reduced HSP70 gene expression in muscle tissue of heat stressed 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 expression of this gene that the result from exposure to heat stress, and as a result, reduce the harmful effects of heat stress. It can be concluded that *H. illucens* meal can be used as nutritional approach for heat stress mitigation in Japanese quail with the HSP70 gene expression indicator.

Declarations

i. Funding 'Not applicable'

ii. Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

iii. Compliance with Ethical Standards

The study was approved by the Ethics Committee of Local Experimental Animals Care Committee, Egyptian Nuclear Research Center.

iv. Consent to participate (include appropriate statements) 'Not applicable'

v. Consent for publication (include appropriate statements) 'Not applicable'

vi. Availability of data and material (data transparency) 'Not applicable'

vii. Code availability (software application or custom code) 'Not applicable'

viii. Authors' contributions

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Figures

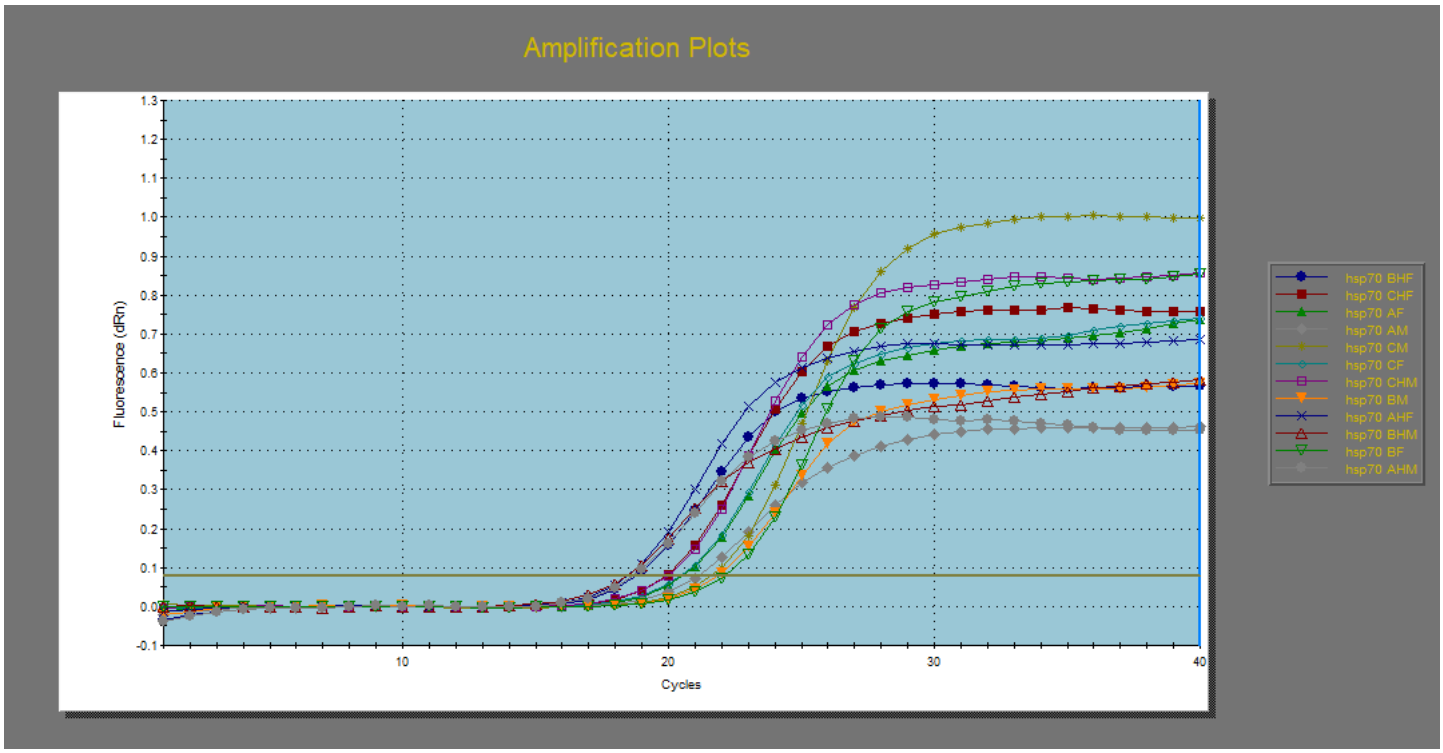

Figure 1

Target HSP70 gene expression in two replacement of SP with *H. Illunces* meal and two treatments groups (exposed and non-exposed to temperatures) of Japanese quill.

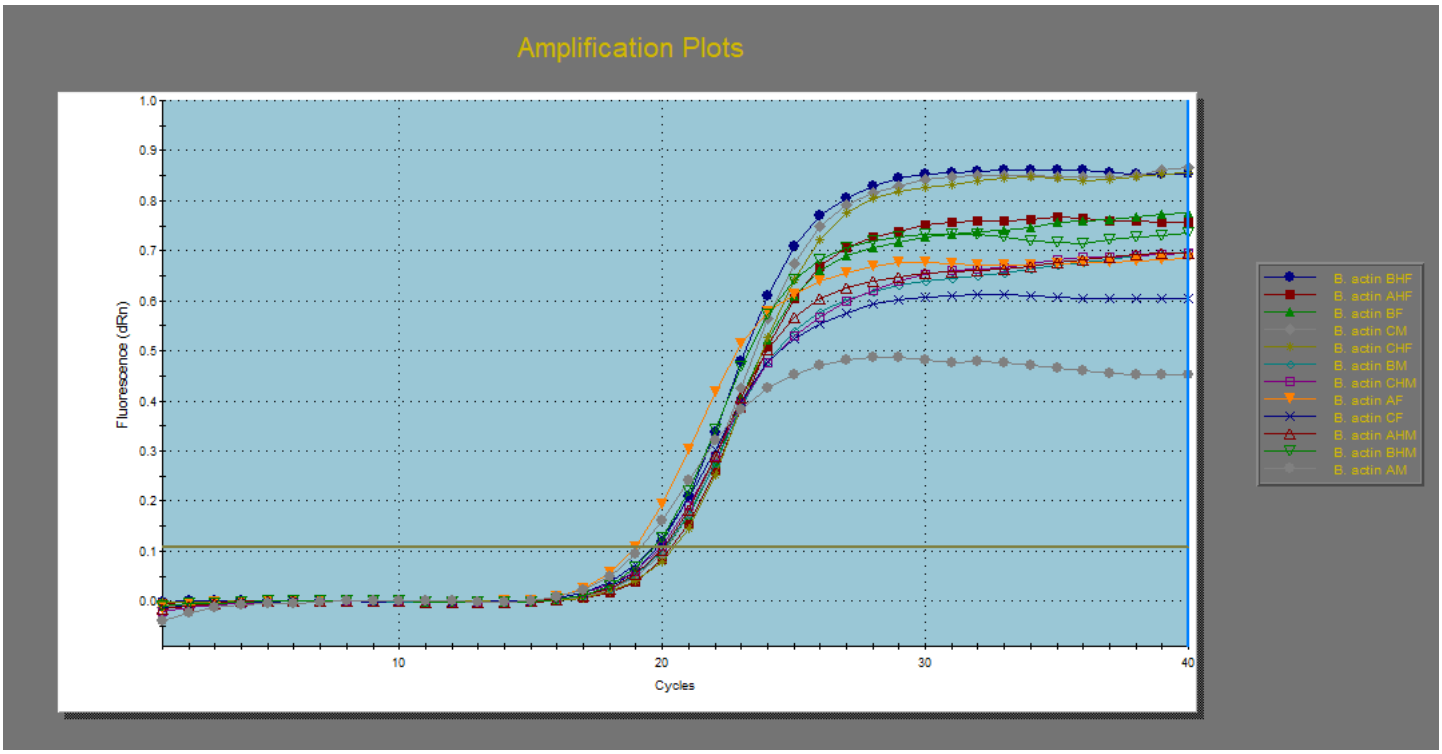

Figure 2

Referance *ß. Actin* gene expression in two replacement of SP with *H. Illunces* meal and two treatments groups (exposed and non-exposed to temperatures) of Japanese quill