Tenebrio molitor and *Zophobas morio* full-fat meals as functional feed additives affect broiler chickens' growth performance and immune system traits

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ABSTRACT This study was conducted to investigate the effect of insect full-fat meals (*Tenebrio molitor* and *Zophobas morio* larvae), added "*on top*" of a complete diet or calculated into diets, on the growth performance, selected blood, and immune system traits of broiler chickens. 1,000 one-day-old female Ross 308 broiler chicks were used in 2 independent experiments. In the first trial, the birds were randomly assigned to 6 treatments, 10 replicate pens per treatment, and 10 birds per pen, i.e., negative control; positive control with salinomycin addition (60 mg/kg diet), and addition of 0.2% and 0.3% of *T. molitor* and *Z. morio* full-fat meals "*on top*". In the second experiment, 4 treatments, 10 replicate pens per treatment, and 10 birds per pen were set, i.e., negative control, positive control with salinomycin addition (60 mg/kg diet), and 0.3% of *T. molitor* and *Z. morio* full-fat meals calculated in the diets. In both trials the supplementation of insects increased the BWG (Exp. 1: $P = 0.024$; Exp. 2: $P = 0.046$) and FI (Exp. 1: $P = 0.022$; Exp. 2: $P = 0.026$), and no negative effect on the FCR was recorded in experiment one ($P = 0.514$), however in second trial insects addition increased FCR values ($P = 0.011$). In addition, in the first trial, groups fed insects and PC comparing to NC decreased the IgY ($P = 0.045$) and IgM, ($P < 0.001$) levels. In the second experiment, IgM levels were also decreased ($P < 0.001$) in groups fed insects comparing to NC. Moreover, in first trial the IgM levels were negatively correlated to the BWG (r = –0.4845) and FI (r = –0.4986), with statistically significant values ($P < 0.001$). In conclusion, the current results confirmed that small amount addition (0.2% and 0.3%) of *T. molitor* and *Z. morio* full-fat meals to the diet of broiler chickens can improve growth performance and change selected the immune system traits.

Key words: insect, mealworm, super mealworm, broiler chicken, immune system

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INTRODUCTION

Insects have been proposed as a sufficient sustainable and alternative source of nutrients for livestock, including poultry (Premalatha et al., 2011; María–José Sánchez–Muros et al., 2014; Józefiak et al., 2016). It should be highlighted that consuming insects is a natural feeding habit for various species, including pig, poultry, and fish (Veldkamp et al., 2012; Makkar et al., 2014; Henry et al., 2015). Among the various species of insects, yellow mealworm (*Tenebrio molitor*; **TM**) and super mealworm larvae (*Zophobas morio*; **ZM**) have been predominantly studied as a source of protein and fat for different animal species due to their qualitative and quantitative amino-acid and fatty-acid profiles (Józefiak et al., 2016; Kierończyk et al., 2018). Presently, insects are not only considered as a nutritional carrier but also as a dietary factor that has a wide spectrum of activities against pathogens due to their ability to synthesize antimicrobial peptides (**AMPs**) (Józefiak and Engberg, 2017). AMPs are small cationic peptides that affect the molecules of innate immunity and have shown a broad spectrum of activity against bacteria, fungi, and viruses (Boman, 1995; Imamura et al., 1999; Thacker, 2013; Józefiak

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and Engberg, 2017). In addition to synthesizing AMPs, insects are a rich source of chitin, whose beneficial effect in increasing the activity of the innate immune system, as well as its antibacterial effects, have been widely studied (Koide, 1998; Esteban et al., 2001; Xu et al., 2013). In addition, Lee et al. (2008) reported that chitin has various biological activities, including immunostimulation. On the other hand, the mechanisms of immunity in birds can be directly affected by several factors, including nutritional ones (Kaiser and Balic, 2015). Numerous studies have reported various alternative protein sources, such as yeast and spray-dried animal plasma, as functional feed additives for broiler chicken, pig, and fish (Van Dijk et al., 2001; Paryad and Mahmoudi, 2008; Pongpet et al., 2016; Shurson, 2017). In most available literature, insects have been studied as alternative protein sources in the broiler chicken diet with various levels of inclusion, i.e., from 1% up to a full replacement of the soybean meal (Ramos–Elorduy et al., 2002; Ballitoc and Sun, 2013; Bovera et al., 2015; Bovera et al., 2016; Biasato et al. 2017). Recently, Józefiak et al. (2018) concluded that the inclusion of small amounts of insect full-fat meals, i.e., from 0.05 to 0.2% in the broiler chicken diet can modulate the microbiota composition of the gastrointestinal tract (**GIT**). In addition, Kierończyk et al. (2018) stated that *T. molitor* oil might be considered a bioactive substance to be used as an alternative to soybean oil, which is commonly used in broiler chicken nutrition, potentially prompting a positive effect on the fatty acid profile of breast muscle and even on the expression of select genes in the liver, such as GIMAP5 which is key regulator of hematopoietic integrity and lymphocyte homeostasis, and APOA1 which is identified as one of the genes responsible for phenotypic fatness variability. Diet composition may modulate immune function in broiler chickens (Kidd, 2004). However, there are no data in the available literature about the effects of full-fat insect meals on the immune system of broiler chickens. Therefore, we hypothesized that small amount additions (0.2 and 0.3%) of insect full-fat meals may improve the growth performance of broiler chickens through the modulation of the immune system traits. The present study was conducted to evaluate the effect of mealworm (*Tenebrio molitor*) and super mealworm larvae (*Zophobas morio*), added in relatively small amounts (0.2 and 0.3%) "*on top*" of a complete diet or calculated into a diet, on the growth performance, various blood parameters and immune system traits of broiler chickens.

MATERIAL AND METHODS

According to Polish law and the EU directive (no 2010/63/EU), the experiments conducted within the study do not require the approval of the Local Ethical Committee for Experiments on animals in Poznań. All procedures and experiments complied with the guidelines and all efforts were made to minimize suffering of the animals.

Table 1. Composition of the basal experimental diets (experiment 1).

Ingredients (g/kg)	1 to 14 D	15 to 35 D
Wheat	487.4	513.4
Rye	100.0	100.0
Soybean meal	207.8	169.5
Rapeseed meal	100.0	100.0
Fish meal	20.0	20.0
Soybean oil	49.9	71.1
Vitamin–mineral premix[a]	3.0	3.0
Monocalcium Phosphate	13.1	6.7
Limestone	8.0	6.8
Salt (NaCl)	1.1	1.3
Sodium carbonate (Na_2CO_3)	2.2	1.7
L-Lysine HCl	2.9	2.4
Methionine 88% liquid	3.1	2.5
L-Threonine	1.5	1.6
Calculated nutritive value (g/kg)		
Crude protein	215.6	200.6
Ether extract	65.4	86.3
Crude fiber	33.1	32.2
Total Phosphorus (P)	7.9	6.3
Calcium (Ca)	8.5	7.0
Methionine	6.1	5.3
Lysine	12.5	11.2
Methionine + Cysteine	9.9	9.0
Threonine	9.1	8.6
AME_N (MJ/kg)	12.56	13.31

[a]Provided the following per kilogram of diet: vitamin A, 11,166 IU; cholecalciferol, 2,500 IU; vitamin E, 80 mg; menadione, 2.50 mg; vitamin B_{12}, 0.02 mg; folic acid, 1.17 mg; choline, 379 mg; D-pantothenic acid, 12.50 mg; riboflavin, 7.0 mg; niacin, 41.67 mg; thiamine, 2.17 mg; D-biotin, 0.18 mg; pyridoxine, 4.0 mg; ethoxyquin, 0.09 mg; Mn (MnO_2), 73 mg; Zn (ZnO), 55 mg; Fe ($FeSO_4$), 45 mg; Cu ($CuSO_4$), 20 mg; I (CaI_2O_6), 0.62 mg; and Se (Na_2SeO_3), 0.3 mg.

Birds and Housing

A total of 1,000 one-day-old female Ross 308 broiler chicks were used in 2 independent experiments at the experimental unit (Piast, Olszowa Experimental Unit, no. 0161, Olszowa, Poland). In the first experiment, birds were randomly assigned into 6 dietary treatment groups, with 10 replicate pens per treatment and 10 birds per replicate pen. In the second trial, birds were assigned into 4 dietary treatment groups, with 10 replicate pens per treatment and 10 birds per replicate pen. The experiments were conducted to investigate the growth performance, selected blood parameters, immune system traits, and selected organ weights. The housing parameters were the same in both experiments and, in accordance with the guidelines, the birds were given 23 h of light and 1 h without light for the first week, followed by 19 h of light and 5 h without from 7 to 21 D of age. From 22 to 35 D of age, the lighting system was similar to that of the first week. The birds were kept in floor pens (1.00 × 1.00 m) for 35 D. birds were vaccinated against Gumboro disease at day 21 (AviPro PRECISE, Lohmann Animal GmbH, Cuxhaven, Germany).

Diets and Feeding Program

The ingredient compositions of the experimental diets are shown in Tables 1 and 2. The birds were fed

Table 2. Composition of the experimental diets (experiment 2).

Ingredients (g/kg)	PC[1], 1 to 14 D	PC, 15 to 35 D	NC[2], 1 to 14 D	NC, 15 to 35 D	TM03[3], 1 to 14 D	TM03, 15 to 35 D	ZM03[4], 1 to 14 D	ZM03, 15 to 35 D
Wheat	482.63	505.09	482.63	505.09	484.03	506.48	484.25	506.71
Rye	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Soybean meal	206.87	168.94	206.87	168.94	203.37	165.44	203.14	165.21
Rapeseed meal	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Fish meal	20.0	20.0	20.0	20.0	20.0	20.0	20.0	2.0
Soybean oil	54.85	78.45	54.85	78.45	53.90	77.50	53.89	77.49
Vitamin–mineral premix[a]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Monocalcium phosphate	15.33	9.93	15.33	9.93	15.32	9.93	15.33	9.94
Limestone	7.13	5.53	7.13	5.53	7.16	5.56	7.15	5.55
Salt (NaCl)	2.38	1.88	2.38	1.88	2.38	1.88	2.38	1.88
Sodium carbonate (Na_2CO_3)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
L-Lysine HCl	2.83	2.55	2.83	2.55	2.85	2.57	2.86	2.58
Methionine 88% liquid	2.4	1.95	2.4	1.95	2.4	1.95	2.41	1.95
L-Threonine	1.59	1.69	1.59	1.69	1.59	1.69	1.59	1.69
Tenebrio molitor full-fat meals	0	0	0	0	3.0	3.0	0	0
Zophobas morio full-fat meals	0	0	0	0	0	0	3.0	3.0
Calculated nutritive value (g/kg)								
Crude protein	223.14	207.97	223.14	207.97	223.10	207.90	223.07	207.92
Ether extract	70.15	93.40	70.15	93.40	70.10	93.35	70.22	93.47
Crude fiber	32.99	32.00	32.99	32.00	32.88	31.89	32.87	31.89
Crude ash	62.04	52.85	62.04	52.85	61.95	52.80	61.95	52.69
Total phosphorus (P)	8.39	7.00	8.39	7.00	8.40	7.02	8.40	6.99
Calcium (Ca)	8.49	6.99	8.49	6.99	8.52	7.03	8.52	7.01
Sodium (Na)	1.61	1.41	1.61	1.41	1.61	1.41	1.61	1.41
Methionine	6.17	5.46	6.17	5.46	6.17	5.44	6.17	5.44
Lysine	12.80	11.58	12.80	11.58	12.79	11.57	12.79	11.57
Methionine + Cysteine	10.18	9.27	10.18	9.27	10.17	9.25	10.17	9.27
Threonine	9.43	8.94	9.43	8.94	9.45	8.90	9.45	8.93
AME_N (MJ/kg)	12.56	13.31	12.56	13.31	12.51	13.26	12.51	13.26
Analyzed chemical composition (g/kg)								
Crude protein	216.00	203.30	214.00	198.00	217.00	203.00	216.00	199.00
Ether extract	71.00	75.00	67.00	81.00	67.00	89.00	67.00	84.00
Gross energy (MJ/kg)	17.74	18.66	17.75	18.63	17.90	18.84	17.95	18.82

[a]Provided the following per kilogram of diet: vitamin A, 11,166 IU; cholecalciferol, 2,500 IU; vitamin E, 80 mg; menadione, 2.50 mg; vitamin B_{12}, 0.02 mg; folic acid, 1.17 mg; choline, 379 mg; D-pantothenic acid, 12.50 mg; riboflavin, 7.0 mg; niacin, 41.67 mg; thiamine, 2.17 mg; D-biotin, 0.18 mg; pyridoxine, 4.0 mg; ethoxyquin, 0.09 mg; Mn (MnO_2), 73 mg; Zn (ZnO), 55 mg; Fe ($FeSO_4$), 45 mg; Cu ($CuSO_4$), 20 mg; I (CaI_2O_6), 0.62 mg; and Se (Na_2SeO_3), 0.3 mg.

[1]NC—Negative control.

[2]PC—positive control − NC + salinomycin addition (60 mg/kg diet).

[3]TM03–NC with 0.3% *T. molitor* full-fat meals.

[4]ZM03–NC with 0.3% *Z. morio* meals full-fat meals.

ad libitum during the entire period of the experiments. The diets were prepared in mash form; all the raw materials were ground by a disc mill (Skiold A/S, Saby, Denmark) at a 2.5-mm disc distance and mixed with any necessary heat treatment. The diets were produced in the Piast Pasze feed mill (Lewkowiec, Poland) according to ISO 9001:2008 procedures. The feed was prepared on a laboratory-scale line equipped with a horizontal double band mixer (Zuptor, Gostyń, Poland) with roller mills (Skiold, Saby, Denmark). Starter diets were offered to all birds from 1 to 14 D of age, and grower diets were offered from 15 to 35 D of age. No exogenous enzymes were added to the diets. In the first experiment, the insect full-fat meals were added "*on top*" of the complete diet, while in the second experiment, the insect full-fat meals were calculated into the diets. Additionally, based on the results of the first experiment, the authors decided to select treatments that showed the best growth performance results.

The experimental treatments used were as following:

Experiment 1: NC (negative control) − no additives; PC (positive control) − NC + salinomycin addition (60 mg/kg diet); TM02 − NC + 0.2% *T. molitor* full-fat meals; ZM02 − NC + 0.2% *Z. morio* full-fat meals; TM03 − NC + 0.3% *T. molitor* full-fat meals; and ZM03 − NC + 0.3% *Z. morio* full-fat meals.

Experiment 2: NC (negative control) − no additives; PC (positive control) − NC + salinomycin addition (60 mg/kg diet); TM03 − NC with 0.3% *T. molitor* full-fat meals; and ZM03 − NC with 0.3% *Z. morio meals* full-fat meals.

Preparation of Insect Full-fat Meals

Both insect species (*T. molitor* and *Z. morio*) used in the current experiments were obtained from a commercial source (HiProMine S.A., Robakowo, Poland), air-dried in an oven (SLN 240, POL–EKO Aparatura, Poland) for 24 h at 50°C, and finely ground (Zelmer motor blocked power 1,900 w, Rzeszów, Poland) to obtain full-fat meals.

Data and Sample Collection

In both experiments, the following variables were analyzed on days 14 and 35 of the experiments, body weight gain (**BWG**) and feed intake (**FI**) were measured, and the feed conversion ratio (**FCR**) was calculated. At the end of the experiments (35 D), 1 bird per replication (10 birds per group) were randomly selected, blood samples were collected after slaughter, and serum was obtained by centrifugation (Micro 220R, Hettich, Tuttlingen, Germany) at 1,000 × *g* at 8°C for 10 min and stored at –20°C until analysis. The following blood parameters were determined: non-esterified fatty acid (**NEFA**), glucose, triglycerides, total cholesterol, total protein (**TP**), and albumin. The methods used for serum analysis are described in the next paragraph.

The concentrations of the selected serum parameters, i.e., immunoglobulin Y (**IgY**), immunoglobulin M (**IgM**), immunoglobulin A (**IgA**), interleukin-2 (**IL-2**), interleukin-6 (**IL-6**) and tumor necrosis factor-alpha (**TNF-α**), were assayed using species-specific commercial ELISA kits for chicken IgY, IgM, and IgA (Abnova, Taipei City, Taiwan), as well as IL-2, IL-6, and TNF-α (Shanghai Sunred Biological Technology Co., Ltd, Shanghai, China); the analyses were done according to the manufacturer's instructions. Each sample was analyzed in duplicates. The birds were killed by cervical dislocation, and after dissection, the internal organ weights were measured. The relative weight of the organs was determined with respect to the live body weight of the birds (% BW). The organs were accurately weighed using a laboratory scale (PS 600/C/2, Radwag, Radom, Poland).

Analyses of Blood Serum Parameters

The concentration of NEFA was measured according to the colorimetric method described by Duncombe (1964). The glucose concentration was analyzed colorimetrically using a Synergy 2 Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT) with a kit reagent (Pointe Scientific, Warsaw, Poland). Total cholesterol and triglycerides were measured using commercial enzymatic kits (Pointe Scientific, Canton, MI), and TP and albumin were determined according to the methods described by Szymeczko et al. (2008).

Chemical Analysis

The amino acid and fatty acid compositions of insect meals were analyzed according to the method described by AOAC (2005) procedures. Dry matter (**DM**), crude protein, ether extract, crude ash, and crude fiber were determined using methods 934.01, 976.05, 920.39, 920.153, and 985.29, respectively. The chitin composition of the insect meals was determined as described by Soon et al. (2018). The compositions of the analyzed insect full-fat meals are presented in Table 3, and the fatty acid profile is shown in Table 4.

Calculations and Statistical Analyses

The internal organ indexes (% of BW) were calculated as the organ weight (g) divided by the live chicken's weight before slaughter (Zhu et al., 2015).

The designs of the experiments were completely randomized, and data were tested using the General Linear Models procedure of SAS software. In the experiments, means were separated using Duncans' tests following one-way ANOVA based on the following equation:

$$y_{ij} = \mu + a_i + \delta_{ij},$$

where y_{ij} is the observed dependent variable; μ is the overall mean; α_i is the effect of treatment; and δ_{ij} is the random error. In cases in which the overall effect was significant, $P \leq 0.05$.

Table 3. Nutrient composition of *Tenebrio molitor* and *Zophobas morio* full-fat meals used in the experiments.

Item	*Tenebrio molitor*	*Zophobas morio*
Dry matter (%)	95.58	96.32
Crude protein (% of DM)	47.0	49.3
Ether extract (% of DM)	29.6	33.6
Crude ash (% of DM)	2.56	2.52
Crude fiber (% of DM)	5.6	5.1
Chitin (% of DM)	8.91	4.59
Calcium (% of DM)	0.05	0.05
Phosphorus (% of DM)	0.72	0.62
Amino acids composition (% of DM)		
Indispensable amino acids		
Arginine	2.51	2.52
Histidine	1.41	1.41
Isoleucine	2.08	2.18
Leucine	3.78	3.56
Lysine	2.65	2.71
Methionine	0.73	0.67
Phenylalanine	1.93	1.82
Threonine	2.51	1.97
Valine	3.33	2.97
Tryptophan	0.47	0.57
Dispensable amino acids		
Alanine	4.17	3.76
Aspartic acid	3.82	3.83
Cysteine	0.58	0.49
Glycine	2.65	2.49
Glutamic acid	6.03	6.40
Proline	3.13	2.68
Serine	2.28	2.23
Tyrosine	3.14	3.30

Table 4. Fatty acid profile of *Tenebrio molitor* and *Zophobas morio* full-fat meals (% DM).

Item	*Tenebrio molitor*	*Zophobas morio*
Saturated fatty acids		
C14:0 Myristic	0.9	0.3
C16:0 Palmitic	6.3	10.5
C18:0 Stearic	1.2	3.1
Total SFA[1]	8.5	14.4
Monounsaturated fatty acids		
C16:1 Palmitoleic	0.6	0.2
C18:1 *c*9 Oleic	13.4	10.3
Total MUFA[2]	14.2	10.8
Polyunsaturated fatty acids		
C18:2 *c*9*c*12 Linoleic, LA	9.2	8.2
C18:3 *c*9*c*12c15 α-Linolenic, LNA	0.4	0.3
Total PUFA[3]	9.6	8.6
n-3	0.4	0.3
n-6	9.2	8.2
n-9	13.4	10.3

[1]SFA—saturated fatty acids.
[2]MUFA—monounsaturated fatty acids.
[3]PUFA—polyunsaturated fatty acids.

The correlation between immunoglobulins concentration and growth performance factors was assessed using Person's correlation on GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA) calculated from the following formula:

$$r_{xy} = \mathrm{cov}\,(\mathrm{x}, \mathrm{y})\,/\mathrm{s_x s_y},$$

where r_{xy} is the Pearson's correlation coefficient, cov (x, y) is the covariance (covariation between x and y), and S_x and S_y are the standard deviations of the x and y cultivars, respectively.

RESULTS

Bird Performance

Experiment 1 The effects of *T. molitor* and *Z. morio* larvae supplementation on the growth performance of broiler chickens are shown in Table 5. From 1 to 14 D, significant differences were observed in the case of BWG and FCR ($P = 0.038$, $P = 0.009$ respectively), and the highest value of BWG was noted in ZM03, with no differences between PC, TM02, ZM02, and TM03. The lowest BWG was in the NC group. The lowest FCR value was observed in the case of PC, ZM02, TM03, and ZM03, while the NC showed the highest value. In the case of FI, no significant differences between all groups were recorded. From 15 to 35 D, significant differences were noted in the case of BWG and FI ($P = 0.044$, $P = 0.012$, respectively). Meanwhile, no differences were found in the case of FCR. The BWG showed the highest value in ZM03, with no differences between the ZM02 and TM03 groups and being the lowest in the NC group. While PC and TM02 did not differ among other treatments. Considering the entire period of the experiment, statistically significant differences were observed in the case of BWG and FI ($P = 0.024$, $P = 0.022$); meanwhile, there were no differences in FCR value. Both *T. molitor* and *Z. morio* larvae additions in each proportion increased the BWG and FI in comparison to NC and PC, with a remarkable difference in the BWG (7.11%) between ZM03 and NC and in the BWG (3.25%) between ZM03 and PC.

Experiment 2 The growth performance results are shown in Table 6. From 1 to 14 D, the BWG showed a variable tendency ($P = 0.062$); the highest value was noticed in TM03, and the lowest occurred in the NC. Meanwhile, no differences were observed in FI and FCR. The FCR values in this period show a trend with the lowest values at TM03 and ZM03. In the second period (from 15 to 35 D), statistically significant differences were observed in all parameters, i.e., BWG, FI, and FCR ($P = 0.027$, $P = 0.035$, and $P < 0.001$, respectively); the highest BWG value was found in TM03 and PC and the lowest was found in NC, while the highest FI value was in TM03. The FCR showed a low value in the PC, with significant differences compared to the other treatments. During the entire period of the experiment, the BWG, FI, and FCR exhibit significant differences between treatments ($P = 0.046$, $P = 0.026$, and $P = 0.011$, respectively). The highest BWG and FI values were observed in TM03, and the lowest was in the NC. The FCR value shows a low value in the PC, with significant differences compared to the other treatments.

Table 5. The effect of dietary supplementation with selected full-fat insect meals on the growth performance of broiler chickens (experiment 1).

	1 to 14 D			15 to 35 D			1 to 35 D		
Item	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g
PC	382[a]	549	1.44[b]	1671[a,b]	2783[a,b]	1.67	2053[a,b]	3332[a,b]	1.62
NC	362[b]	551	1.52[a]	1609[b]	2703[b]	1.68	1971[b]	3254[b]	1.65
TM02	372[a,b]	546	1.47[a,b]	1663[a,b]	2794[a,b]	1.68	2035[a,b]	3340[a,b]	1.64
ZM02	382[a]	549	1.44[b]	1706[a]	2852[a]	1.67	2088[a]	3401[a]	1.63
TM03	382[a]	536	1.40[b]	1722[a]	2897[a]	1.68	2104[a]	3433[a]	1.63
ZM03	388[a]	543	1.40[b]	1734[a]	2919[a]	1.68	2122[a]	3462[a]	1.63
SEM[1]	2.53	1.97	0.01	12.71	19.88	0.01	14.27	19.81	0.01
P-value	*0.038*	*0.301*	*0.009*	*0.044*	*0.012*	*0.908*	*0.024*	*0.022*	*0.514*

[a,b]Means within a column with no common superscripts differ significantly ($P < 0.05$).
[1]SEM—standard error of the mean.
Data are means of 10 replicate pens with 10 birds per pen.

Table 6. The effect of dietary supplementation with selected full-fat insect meals on the growth performance of broiler chickens (experiment 2).

	1 to 14 D			15 to 35 D			1 to 35 D		
Item	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g
PC	391	543	1.39	1743[a]	2741[b]	1.57[b]	2134[a,b]	3283[b]	1.54[b]
NC	388	541	1.40	1688[b]	2716[b]	1.61[a]	2076[b]	3257[b]	1.57[a]
TM03	408	549	1.35	1739[a]	2831[a]	1.63[a]	2148[a]	3380[a]	1.57[a]
ZM03	401	541	1.35	1693[b]	2763[a,b]	1.63[a]	2094[a,b]	3304[a,b]	1.58[a]
SEM[1]	18.05	13.74	0.06	49.72	87.28	0.03	61.99	89.94	0.03
P-value	*0.062*	*0.458*	*0.196*	*0.027*	*0.035*	*<0.001*	*0.046*	*0.026*	*0.011*

[a,b]Means within a column with no common superscripts differ significantly ($P < 0.05$).
[1]SEM—standard error of the mean.
Data are means of 10 replicate pens with 10 birds per pen.

Table 7. Weights of selected internal organs relative to live body weight of broilers fed selected full-fat insect meals (% of BW) (experiment 1).

Item	Liver	Pancreas	Spleen	Bursa of Fabricius
PC	2.29	0.25	0.10	0.15[b]
NC	2.38	0.24	0.11	0.20[a]
TM02	2.42	0.27	0.11	0.15[b]
ZM02	2.55	0.23	0.11	0.14[b]
TM03	2.27	0.25	0.12	0.15[b]
ZM03	2.28	0.25	0.11	0.14[b]
SEM[1]	0.04	0.01	<0.01	0.01
P-value	*0.203*	*0.294*	*0.747*	*0.049*

[a,b]Means within a column with no common superscripts differ significantly ($P < 0.05$).
[1]SEM—standard error of the mean.
Data are means of 10 replicate pens with 1 bird per pen.

Weight of Internal Selected Organs

Experiment 1 The weights of selected internal organs are shown in Table 7. There were no differences in the relative weights of the liver, pancreas, and spleen to the BW, except the bursa of Fabricius, which was affected by the dietary treatment. Significant differences ($P = 0.049$) were noted, and both full-fat insect meals and salinomycin reduced the weight in comparison to the NC.

Experiment 2 No statistically significant differences were observed in the relative weights of the internal organs, i.e., liver, pancreas, spleen, bursa of Fabricius, and thymus (data not shown).

Selected Blood Parameters

Experiments 1 and 2 The concentrations of the selected blood parameters are shown in Table 8. The NEFA values showed statistically significant differences ($P = 0.035$), and the TM02, ZM02, and ZM03 decreased the level of the NEFA in comparison to the PC, while there were no differences in TM03 and NC. The glucose level was not significantly affected. However, a tendency was recorded ($P = 0.072$), where the lowest value was observed in the NC. No differences were observed in total cholesterol, triglycerides, albumin, and TP levels.

The results of the second experiment indicated that the addition of full-fat insect meals generally affected the content of TP and albumin with statistically significant differences ($P < 0.001$ and $P = 0.005$, respectively); the highest value occurred with the addition of ZM03, while the lowest value of albumin was in the negative and positive controls. Triglycerides content was not affected by any of the experimental diets, while the content of total cholesterol was statistically significant

Table 8. The effect of dietary supplementation with selected full-fat insect meals on the concentrations of selected blood parameters.

Item	NEFA[1] (mmol/L)	Total protein (g/dL)	Albumin (g/dL)	Glucose (mg/dL)	TG[2] (mg/dL)	TCh[3] (mg/dL)
Experiment 1						
PC	0.59[a]	4.66	2.67	227.80	83.34	139.29
NC	0.55[a,b]	4.50	3.08	211.23	77.17	138.14
TM02	0.50[b]	4.62	2.69	217.03	70.55	141.16
ZM02	0.51[b]	4.61	3.24	227.83	69.84	138.95
TM03	0.54[a,b]	4.56	2.55	236.28	67.54	140.23
ZM03	0.51[b]	4.72	4.19	227.06	61.17	141.60
SEM[3]	0.07	0.28	1.63	18.21	18.42	13.84
P-value	*0.035*	*0.699*	*0.392*	*0.072*	*0.224*	*0.995*
Experiment 2						
PC	0.66[b]	4.82[b]	2.64[b]	208.13	99.32	123.92[b]
NC	0.77[a]	5.04[a]	2.55[b]	216.43	104.50	123.18[b]
TM03	0.69[b]	5.21[a]	2.67[a,b]	216.88	98.47	135.68[a]
ZM03	0.64[b]	5.15[a]	2.76[a]	224.17	100.57	116.59[c]
SEM[4]	0.09	0.24	0.16	16.74	17.69	6.90
P-value	*<0.001*	*<0.001*	*0.005*	*0.072*	*0.779*	*<0.001*

[a–c]Means within a column with no common superscripts differ significantly ($P < 0.05$).
[1]Non-esterified fatty acid.
[2]Triglycerides.
[3]Total cholesterol.
[4]SEM—standard error of the mean.
Data are means of 10 replicate pens with 1 birds per pen.

and decreased at the addition of ZM03, with the highest content observed in the TM03 group. There was a noted tendency of the glucose level ($P = 0.072$), and it is remarkable that the addition of full-fat insect meals increases the plasma glucose level in comparison to the NC and PC. In addition, the NEFA content was affected by the dietary treatments, and statistically significant differences ($P < 0.001$) were observed, as the full-fat insect meals and salinomycin decreased the NEFA level in comparison to the NC.

Immune Parameters Status

Experiments 1 and 2 The effects of the dietary treatments on the immunological serum parameters are shown in Table 9. In the first experiment, the addition of insect full-fat meals added "*on top*" as well as the salinomycin decreased significantly the IgY ($P = 0.045$) and IgM, ($P < 0.001$) levels. Meanwhile, a tendency was noted in the IgA level ($P = 0.061$) with a decrease in the addition of full-fat insect meals and salinomycin. The IL-2 was significantly affected ($P < 0.001$) by the dietary treatment, in which the highest value was observed in TM03 and the lowest was observed in the NC. TNF-α was also significantly affected ($P = 0.016$), by which ZM03 and TM03 increased its value.

In the second experiment, the IgM was also significantly affected ($P < 0.001$) by the addition of insect full-fat meals (calculated in diet), and level of IgM in all groups was statistically decreased ($P < 0.001$) in comparison to NC group. However, no effect was noted on the other parameters (IgY, IgA, IL-2, TNF-α, and IL-6).

Correlation

The correlation between immunoglobulin concentration and FI and BWG in the first experiment is shown in Table 10. The IgM was negatively correlated to the BWG (r = –0.4845) and the FI (r = –0.4986), with statistically significant values ($P < 0.001$). Meanwhile, no correlation was recorded between IgY, IgA and BWG, FI.

DISCUSSION

A total of 2 independent experiments were conducted to examine the effects of relatively small inclusions (0.2 to 0.3%) of *T. molitor* and *Z. morio* full-fat meals in the diets on the growth performance, internal organs, selected blood, and immune system traits of the broiler chickens.

In the first experiment, the inclusion of ZM03 showed the best results with 3.25% higher BWG than PC and 7.11% higher BWG than NC ($P = 0.024$). Islam and Yang (2016) also observed that the implementation of a relatively small amount, i.e., 0.4% *T. molitor* and *Z. morio*, increased the BWG of broiler chickens; however, their diets were enriched by *Lactobacillus plantarum* and *Saccharomices cerevisiae*. It should be emphasized that in the current study, only insect full-fat meals, without any probiotics or other feed additives, were used in the basal diets, and the impact of full-fat insect meals was considered as an experimental factor. In addition, the results of Ballitoc and Sun (2013) showed that 1% addition of *T. molitor* full-fat meals to the broiler chicken diet increase the growth performance. The results of the present study are in line with the

Table 9. The effect of dietary supplementation with selected full-fat insect meals on immunological serum parameters in broilers.

Treatments	IgY[1] (ng/mL)	IgA[2] (ng/mL)	IgM[3] (ng/mL)	IL-2[4] (ng/mL)	TNF-α[5] (ng/mL)	IL-6[6] (ng/mL)
Experiment 1						
PC	70.74[c]	64.28	101.21[b]	55.69[b,c]	260.93[b]	277.83
NC	104.81[a]	88.14	147.11[a]	33.83[c]	271.86[b]	321.02
TM02	83.74[a–c]	71.84	121.17[b]	44.70[c]	269.33[b]	287.44
ZM02	79.75[b,c]	66.45	108.87[b]	78.37[a,b]	281.24[b]	288.86
TM03	90.05[a–c]	65.82	102.43[b]	96.54[a]	384.78[a]	344.61
ZM03	95.34[a,b]	68.42	98.92[b]	69.87[b]	325.15[a,b]	303.09
SEM[1]	31.03	23.93	34.44	22.14	74.40	76.12
P-value	*0.045*	*0.061*	*<0.001*	*<0.001*	*0.016*	*0.553*
Experiment 2						
PC	115.63	67.58	95.26[b]	75.57	277.04	299.82
NC	139.35	80.93	134.24[a]	64.30	418.03	314.68
TM03	118.26	69.62	78.66[b]	61.14	364.47	292.06
ZM03	113.75	77.28	81.02[b]	60.62	423.55	329.06
SEM[7]	63.00	25.44	33.58	23,00	145.93	58.78
P-value	*0.640*	*0.412*	*<0.001*	*0.540*	*0.226*	*0.637*

[a–c]Means within a column with no common superscripts differ significantly ($P < 0.05$).
[1]Immunoglobulin Y.
[2]Immunoglobulin A.
[3]Immunoglobulin M.
[4]Interleukin-2.
[5]Tumor necrosis factor-alpha.
[6]Interleukin-6.
[7]SEM—standard error of the mean.
Data are means of 10 replicate pens with 1 birds per pen.

Table 10. Pearson's correlation coefficients between serum immunoglobulins concentration and BWG, FI of broiler chicken fed selected full-fat insect meals (experiment 1).

Immunoglobulins	Parameters	Correlation coefficient (r)	*P* value
IgY[1]	BWG	–0.1506	*0.318*
IgY	FI	–0.08503	*0.574*
IgA[2]	BWG	–0.1446	*0.327*
IgA	FI	–0.4986	*0.189*
IgM[3]	BWG	–0.4845	*<0.001*
IgM	FI	–0.4986	*<0.001*

[1]Immunoglobulin Y.
[2]Immunoglobulin A.
[3]Immunoglobulin M.

experiment conducted by Biasato et al. (2017), in which the inclusion of 5 to 15% of the *T. molitor* meal in the diet improved the growth performance parameters. On the other hand, Biasato et al. (2016) noticed that the growth performance in fast-growing and intermediate-growing chickens was not affected when birds were fed *T. molitor* added to diets at inclusion levels from 5 to 10%. A total replacement of the soybean meal with *T. molitor* meal did not affect the performance of the broiler chicken from 30 to 62 D (Bovera et al., 2016). The causes of various data obtained in the available literature should be connected with the different nutritional value of insects, which is species- and life stage-dependent. Moreover, the rearing medium of invertebrates is not meaningless, as it determines the quantity and quality of protein and fat in their body (Tschirner and Simon, 2015). Furthermore, the nutrient content of insects is frequently affected by meal preparation processes (Dreassi et al., 2017; Makkar et al., 2014). In addition, some authors have reported that the drying process affects the chemical composition of insects (Lenaerts et al., 2018; Melis et al., 2018). From this point of view, it is the main problem during the comparison of various results when the experimental factor is not unified, and more data are needed to understand the mode of action.

According to Józefiak and Engberg (2017), insect proteins are a potential source of AMPs that can be applied to livestock production. It has been well documented that insects synthesize AMPs constitutively in their body (Boman, 1995; Imamura et al., 1999; Thacker, 2013; Józefiak and Engberg, 2017); therefore, the positive growth performance results in both experiments upon inclusion of insect full-fat meals could be related to the function of AMPs.

In both experiments, the FI was increased in the full-fat insect meals treatments in comparison with NC and PC. According to Ballitoc and Sun (2013) and Islam and Yang, (2016), the inclusion of *T. molitor* to the diet of broiler chickens can increase the feed palatability and this could be due to the attractive smell after processing. On the other hand, Bovera et al. (2016) did not find any differences in the FI value when the inclusion level of *T. molitor* was 30% of the diet. In the first trial, FCR value was not affected by any of the dietary treatments, these results are in line of the studies conducted by Ramos–Elorduy et al. (2002), Ballitoc and Sun (2013) and Bovera et al. (2016). However, in the second experiment, the FCR values were negatively affected by the inclusion of full-fat insect meals to the basal diets. It is difficult to explain why the addition

of full-fat insect meals caused higher FCR values which might have been due to the increase in FI.

In both experiments, no significant effect on internal organ weights was observed, except the bursa of Fabricius size ($P = 0.049$) in the first experiment, which was lowered by full-fat insect meals and salinomycin supplementation. This finding is in line with the study done by Islam and Yang (2016). Bursa of Fabricius is a primary lymphoid organ in birds that plays a fundamental role in the differentiation of B-lymphocytes (Schat and Skinner, 2014). The size of the bursa of Fabricius can be directly affected by several pathologic conditions (infectious diseases, mycotoxins, etc.) (Cazaban et al., 2015). In the first experiment, the size was lowered by both insect species and PC. The reason for the decrease of the bursa size can be due to the presence of AMPs synthesized by insects, which are characterized by a wide spectrum of the antimicrobial mode of action (Boman, 1995; Imamura et al., 1999; Thacker, 2013; Józefiak and Engberg, 2017).

The biochemical parameters of blood are crucial traits to assess the health status and performance of animals, including poultry (Milner et al., 2003; Lumej, 2008). Total protein and albumin concentration are 2 useful factors to evaluate the body condition of poultry (Piotrowska et al., 2011). Plasma proteins play a fundamental role in body homeostasis maintenance. In the first experiment, the TP level was not affected, while in the second experiment, the insects showed a higher value of protein level. Albumin is the most favorable source of amino acids for protein synthesis (Filipović et al., 2007). In this study, the albumin level was not affected in the first experiment; these findings were in accordance with those of Biasato et al. (2016). However, in the second experiment, the insect addition increased the albumin level. The changes in the TP and albumin level could be attributed to the properties of chitin contained in the insect meals (Biasato et al., 2016). On the other hand, other authors did not find any differences in the TP and albumin levels when the total replacement of soybean meal by *T. molitor* was performed (Brovera et al., 2016; Biasato et al., 2017).

Chitin is a major aminopolysaccharide constituent of shells of arthropods, such as crabs, shrimps, and insects (Furda, 1983; Hossain and Blair, 2007). In the present study, during the analysis of *T. molitor* and *Z. morio* full-fat meals, the chitin content showed 8.91% of DM and 4.59% of DM, respectively. Furthermore, chitin was described to reduce total cholesterol and triglycerides serum levels in broiler chickens (Hossain and Blair, 2007). The serum total cholesterol and triglycerides levels were not affected in the first experiment. This is in line with the results of the studies conducted by Bovera et al. (2015) and Biasato et al. (2016). However, total cholesterol was lowered in the second experiment by ZM03, and this could be related to the chitin effect. The level of NEFA increases in blood when the glucose level decreases, which induces lipolysis in the adipose tissue and causes the fatty acids to become the major energy source used by most tissues (Brooks et al., 2016). In both experiments, the addition of insect full-fat meals significantly reduced the level of NEFA. The glucose level showed a tendency to reach a high level during the addition of insect full-fat meals. This could be explained by the high feed intake in the ZM and TM treatment groups. This phenomenon does not seem to be negative on the birds. Previous research done by Van der et al. (1999) showed that the increase of NEFA and decrease in glucose levels is a consequence of feed withdrawal.

The chitin was described as the substance that stimulates the immune response in vertebrates (Esteban et al., 2001; Lee et al., 2008). Furthermore, Islam and Yang (2016) noted that the immunoglobulin levels of broiler chicks were increased after the supplementation of 0.4% of *T. molitor* and *Z. morio* probiotics which were obtained by fermenting the insect meals with *L. plantarum* and *S. cerevisiae*. Their findings indicated that IgG and IgA levels increased in response to *T. molitor* and *Z. morio* supplementation. Immunoglobulins act as antibodies, and they are synthesized in response to antigens (Lumej, 2008).

This contrasts with the present study, where no birds' challenge trials were performed, and the levels of IgM and IgY were significantly high in the NC in comparison to the other treatments with insect inclusion and PC. This clearly indicates that the inclusion of insect full-fat meals may have a significant impact on the immune response in broilers. The decreased levels of immunoglobulin classes IgM and IgY in the experimental groups fed full-fat insect meals could be explained by the antimicrobial effects of insect components such as AMPs and chitin. Józefiak et al. (2018) found that the inhibition of possibly pathogenic bacteria and the positive modulation of microbiota in the broiler chicken GIT could be due to full-fat insect meals, potentially similar to the effects of ionophore coccidiostats, i.e., salinomycin. Furthermore, the results of the present study showed that the addition of a relatively small full-fat insect meal may decrease immunoglobulin levels, similar to salinomycin. IgA is abundant in the seromucous secretions such as the GIT, at this site, IgA serves as the first line of defense against many bacteria and viruses (Pleass et al., 2001). In both experiments, the lack of an effect on immunoglobulin class IgA levels in groups with salinomycin addition and groups supplemented with full-fat insect meals could be evidence of no ongoing GIT inflammation. AMPs are involved in the first line of immune defense and possess inhibitory activity against infectious agents through different mechanisms (Dutta and Das, 2016; Xia et al., 2018). Therefore, the immunoglobulin levels in our study could also be related to the insects' AMPs against infectious pathogens. Furthermore, a decrease of IgM secretion correlates with an increase in BWG ($r = –0.4845$) and the FI ($r = –0.4986$) in the first experiment, in which the health status of the birds could be explained. In our study, IL-6 was not affected by

dietary treatments. However, IL-2 was increased in the TM03, and TNF-α was high in the TM03 and ZM03 treatments, which may be related to the insects' AMPs.

CONCLUSIONS

The addition of relatively small amounts of insect full-fat meals to the diets of broiler chicken in 2 independent experiments showed different effects on their biochemical blood parameters, such as TP, albumin, and total cholesterol. However, the same effects were noticed in terms of broiler chicken growth performance, as well as the immune system traits expressed in lower IgM and IgY. We concluded that low inclusion, i.e., 0.2% or 0.3%, of *T. molitor* and *Z. morio* full-fat meals increased the growth performance and changed the immune system traits. Furthermore, the mechanisms by which insect full-fat meals function remain unknown, and further studies are needed to investigate them.

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REFERENCES

AOAC International. 2005. Official Methods of Analysis of AOAC International. 18th ed. W. Hortwitz, and G. W. Latimer, Jr., eds. AOAC Int., Gaithersburg, MD.

Ballitoc, D. A., and S. Sun. 2013. Ground yellow mealworms (*Tenebrio molitor L.*) feed supplementation improves growth performance and carcass yield characteristics in broilers. Open Sci. Reposit. Agric. e23050425.

Biasato, I., M. De Marco, L. Rotolo, M. Renna, C. Lussiana, S. Dabbou, M. T. Capucchio, E. Biasibetti, P. Costa, F. Gai, and L. Pozzo. 2016. Effects of dietary *Tenebrio molitor* meal inclusion in free-range chickens. J. Anim. Physiol. An. N. 100:1104–1112.

Biasato, I., L. Gasco, M. De Marco, M. Renna, L. Rotolo, S. Dabbou, M. T. Capucchio, E. Biasibetti, M. Tarantola, C. Bianchi, and L. Cavallarin. 2017. Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology. Anim. Feed Sci. Tech. 234:253–263.

Boman, H. G. 1995. Peptide antibiotics and their role in innate immunity. Annu. Rev. Immunol. 13:61–92.

Bovera, F., R. Loponte, S. Marono, G. Piccolo, G. Parisi, V. Iaconisi, L. Gasco, and A. Nizza. 2016. Use of *Tenebrio molitor* larvae meal as protein source in broiler diet: effect on growth performance, nutrient digestibility, and carcass and meat traits. J. Anim. Sci. 94:639–647.

Bovera, F., G. Piccolo, L. Gasco, S. Marono, R. Loponte, G. Vassalotti, V. Mastellone, P. Lombardi, Y. Attia, and A. Nizza. 2015. Yellow mealworm larvae *(Tenebrio molitor*, L.) as a possible alternative to soybean meal in broiler diets. Br. Poult. Sci. 56:569–575.

Brooks, M. A., J. L. Grimes, K. E. Lloyd, K. Krafka, A. Lamptey, and J. W. Spears. 2016. Chromium propionate in broilers: effect on insulin sensitivity. Poult. Sci. 95:1096–1104.

Cazaban, C., N. Majo Masferrer, R. Dolz Pascual, M. Nofrarias Espadamala, T. Costa, and Y. Gardin. 2015. Proposed bursa of Fabricius weight to body weight ratio standard in commercial broilers. Poult. Sci. 94:2088–2093.

Dreassi, E., A. Cito, A. Zanfini, L. Materozzi, M. Botta, and V. Francardi. 2017. Dietary fatty acids influence the growth and fatty acid composition of the yellow mealworm *Tenebrio molitor* (Coleoptera: *Tenebrionidae*). Lipids. 52:285–294.

Duncombe, W. G. 1964. The colorimetric micro-determination of non-esterified fatty acids in plasma. Clin. Chim. Acta. 9:122–125.

Dutta, P., and S. Das. 2016. Mammalian antimicrobial peptides: promising therapeutic targets against infection and chronic inflammation. Curr. Top. Med. Chem. 16:99–129.

Esteban, M. A., A. Cuesta, J. Ortuno, and J. Meseguer. 2001. Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system. Fish Shellfish Immunol. 11:303–315.

Filipović, N., Z. Stojević, S. Milinković–Tur, B. Beer Ljubić, and M. Zdelar–Tuk. 2007. Changes in concentration and fractions of blood serum proteins of chickens during fattening. Vet. Arhiv. 77:319–326.

Furda, I. 1983. Aminopolysaccharides — their potential as dietary fiber. Acs. Symp. Ser. 214:105–122.

Henry, M., L. Gasco, G. Piccolo, and E. Fountoulaki. 2015. Review on the use of insects in the diet of farmed fish: past and future. Anim. Feed Sci. Technol. 203:1–22.

Hossain, S. M., and R. Blair. 2007. Chitin utilisation by broilers and its effect on body composition and blood metabolites. Br. Poult. Sci. 48:33–38.

Imamura, M., S. Wada, N. Koizumi, T. Kadotani, K. Yaoi, R. Sato, and H. Iwahana. 1999. *Acaloleptins A.*: inducible antibacterial peptides from larvae of the beetle, *Acalolepta luxuriosa.* Arch. Inscet Biochem. 40:88–98.

Islam, M. M., and C. J. Yang. 2016. Efficacy of mealworm and super mealworm larvae probiotics as an alternative to antibiotics challenged orally with *Salmonella* and *E. coli* infection in broiler chicks. Poult. Sci. 96:27–34.

Józefiak, A., B. Kierończyk, M. Rawski, J. Mazurkiewicz, A. Benzertiha, P. Gobbi, S. Nogales-Mérida, S. Świątkiewicz, and D. Józefiak. 2018. Full-fat insect meals as feed additive — the effect on broiler chicken growth performance and gastrointestinal tract microbiota. J. Anim. Feed Sci. 27:131–139.

Józefiak, A., and R. M. Engberg. 2017. Insect proteins as a potential source of antimicrobial peptides in livestock production. J. Anim. Feed Sci. 26:87–99.

Józefiak, D., A. Józefiak, B. Kierończyk, M. Rawski, S. Świątkiewicz, J. Długosz, and R. M. Engberg. 2016. Insects — a natural nutrient source for poultry — a review. Ann. Anim. Sci. 16: 297–313.

Kaiser, P., and A. Balic. 2015. The avian immune system. Pages 403–418 in Sturkie's Avian Physiology. 6th ed. G. C. Whittow, ed. Academic Press, San Diego, CA.

Kidd, M. T. 2004. Nutritional modulation of immune function in broilers. Poult. Sci. 83:650–657.

Kierończyk, B., M. Rawski, A. Józefiak, J. Mazurkiewicz, S. Świątkiewicz, M. Siwek, M. Szumacher–Strabel, A. Cieślak, A. Benzertiha, and D. Józefiak. 2018. Effects of replacing soybean oil with selected insect fats on broilers. Anim. Feed Sci. Tech. 240:170–183.

Koide, S. S. 1998. Chitin–chitosan: properties, benefits and risks. Nutr. Res. 18:1091–1101.

Lee, C. G., Da C. A. Silva, J. Y. Lee, D. Hartl, and J. A. Elias. 2008. Chitin regulation of immune responses: an old molecule with new roles. Curr. Opin. Immunol. 20:684–689.

Lenaerts, S., M. Van Der Borght, A. Callens, and L. Van Campenhout. 2018. Suitability of microwave drying for mealworms (*Tenebrio molitor*) as alternative to freeze drying: impact on nutritional quality and colour. Food Chem. 254:129–136.

Lumej, J. T. 2008. Avian clinical biochemistry. Pages 839–872 in Clinical Biochemistry of Domestic Animals. J. J. Kaneko, J. W. Harwey, and M. L. Bruss, eds. Elsevier Academic Press, Oxford, UK.

Makkar, H. P., G. Tran, V. Heuzé, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Tech. 197:1–33.

Melis, R., A. Braca, G. Mulas, R. Sanna, S. Spada, G. Serra, M. L. Fadda, T. Roggio, S. Uzzau, and R. Anedda. 2018. Effect of freezing and drying processes on the molecular traits of edible yellow mealworm. Innov. Food Sci. Emerg. Technol. 48:138–149.

Milner, J. M., A. Stien, R. J. Irvine, S. D. Albon, R. Langvatn, and E. Ropstad. 2003. Body condition in Svalbard reindeer and the use of blood parameters as indicators of condition and fitness. Can. J. Zool. 81:1566–1578.

Paryad, A., and M. Mahmoudi. 2008. Effect of different levels of supplemental yeast (*Saccharomyces cerevisiae*) on performance, blood constituents and carcass characteristics of broiler chicks. Afr. J. Agr. Res. 3:835–842.

Piotrowska, A., K. Burlikowska, and R. Szymeczko. 2011. Changes in blood chemistry in broiler chickens during the fattening period. Folia Biol. (Praha). 59:183–187.

Pleass, R. J., T. Areschoug, G. Lindahl, and J. M. Woof. 2001. Streptococcal IgA-binding proteins bind in the Cα2-Cα3 interdomain region and inhibit binding of IgA to human CD89. J. Biol. Chem. 276:8197–8204.

Pongpet, J., S. Ponchunchoovong, and K. Payooha. 2016. Partial replacement of fishmeal by brewer's yeast (*Saccharomyces cerevisiae*) in the diets of Thai Panga (*Pangasianodon hypophthalmus*× *Pangasius bocourti*). Aquacult. Nutr. 22:575–585.

Premalatha, M., T. Abbasi, T. Abbasi, and S. A. Abbasi. 2011. Energy-efficient food production to reduce global warming and ecodegradation: the use of edible insects. Renew. Sust. Energ. Rev. 15:4357–4360.

Ramos-Elorduy, J., E. A. González, A. R. Hernández, and J. M. Pino. 2002. Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. J. Econ. Entomol. 95:214–220.

Sánchez-Muros, M. J., F. G. Barroso, and F. Manzano-Agugliaro. 2014. Insect meal as renewable source of food for animal feeding: a review. J. Clean. Prod. 65:16–27.

Schat, K. A., and M. A. Skinner. 2014. Avian immunosuppressive diseases and immune evasion. Pages 275–297 in Avian Immunology. F. Davison, B. Kaspers, and K. A. Schat, eds. Academic press and Elsevier Ltd, London, U.K.

Shurson, G. C. 2017. Yeast and yeast derivatives in feed additives and ingredients: sources, characteristics, animal responses, and quantification methods. Anim. Feed Sci. Tech. 235:60–76.

Soon, C. Y., Y. B. Tee, C. H. Tan, A. T. Rosnita, and A. Khalina. 2018. Extraction and physicochemical characterization of chitin and chitosan from Zophobas morio larvae in varying sodium hydroxide concentration. Int. J. Boil. Macromol. 108:135–142.

Szymeczko, R., W. Kapelański, A. Piotrowska, J. Dybała, M. Bogusławska–Tryk, K. Burlikowska, I. Hertig, M. Sassek, E. Pruszyńska–Oszmałek, and P. Maćkowiak. 2008. Changes in the content of major proteins and selected hormones in the blood serum of piglets during the early postnatal period. Folia Biol. 57:97–103.

Thacker, P. A. 2013. Alternatives to antibiotics as growth promoters for use in swine production: a review. J. Anim. Sci. Biotechnol. 4:35.

Tschirner, M., and A. Simon. 2015. Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. J. Insects Food Feed. 1:249–259.

Van der Wal, P. G., H. G. Reimert, H. A. Goedhart, B. Engel, and T. G. Uijttenboogaart. 1999. The effect of feed withdrawal on broiler blood glucose and nonesterified fatty acid levels, postmortem liver pH values, and carcass yield. Poult. Sci. 78:569–573.

Van Dijk, A. J., H. Everts, M. J. A. Nabuurs, R. J. C. F. Margry, and A. C. Beynen. 2001. Growth performance of weanling pigs fed spray-dried animal plasma: a review. Livest. Prod. Sci. 68:263–274.

Veldkamp, T., G. van Duinkerken, A. van Huis, C. M. M. Lakemond, E. Ottevanger, G. Bosch, and M. A. J. S. van Boekel. 2012. Insects as a sustainable feed ingredient in pig and poultry diets − a feasibility study. in Rapport 638, Wageningen Livestock Research, https://www.wur.nl/upload_mm/2/8/0/f26765b9-98b2-49a7-ae43-5251c5b694f6_234247%5b1%5d.

Xia, X., L. Cheng, S. Zhang, L. Wang, and J. Hu. 2018. The role of natural antimicrobial peptides during infection and chronic inflammation. Antonie Van Leeuwenhoek. 111:5–26.

Xu, Y., B. Shi, S. Yan, T. Li, Y. Guo, and J. Li. 2013. Effects of chitosan on body weight gain, growth hormone and intestinal morphology in weaned pigs. Asian Austral. J. Anim. 26:1484–1489.

Zhu, W., D. Li, J. Wang, H. Wu, X. Xia, W. Bi, H. Guan, and L. Zhang. 2015. Effects of polymannuronate on performance, antioxidant capacity, immune status, cecal microflora, and volatile fatty acids in broiler chickens. Poult. Sci. 94:345–352.