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THE USE OF THE LARVAE OF *Zophobas morio* AND *Galleria mellonella* IN FEEDING GROWING PIGS

Roman Vladimirovich NEKRASOV[1], Magomed Gazievich CHABAEV[1], Elena Yurievna TSIS[1], Darya Alexandrovna NIKANOVA[2], Gennady Anatolyevich IVANOV[3]

[1] Department of feeding of agricultural animals, Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst, Dubrovitsy, Podolsk urban district, Moscow region, Russia.
[2] Laboratory of microbiology, Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst, Dubrovitsy, Podolsk urban district, Moscow region, Russia.
[3] Limited liability company «NordTechSad», Novodvinsk, Arkhangelsk region, Russia.

Corresponding author:
Roman Vladimirovich Nekrasov
nek_roman@mail.ru

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Abstract

Insects represent a promising alternative source to more expensive components of the feeding regimen of farm animals for meat production. With this aim in mind, we conducted this experiment to investigate the effects of dried larvae of darkling beetles (*Zophobas morio* L.) and larvae of wax moths (*Galleria mellonella* L.) on growth performance, nutrient digestibility and blood profiles in growing piglets. A total of 27 crossbred piglets ((LWxL)xD), 39 days of age and 14.39±0.19 kg of body weight) were randomly divided into three groups (n=9 per group) based on gender and body weight. We substituted part of a fishmeal and a grain with 2.5% of dried larvae of darkling beetles (*Zophobas morio* L.) or with 3.0% of dried larvae of wax moths (*Galleria mellonella* L.). The replacement of fishmeal with insect biomass in the feeding regimen of the pigs did not change growth rate among groups and did not altered the digestibility of dry matter, crude protein and crude fiber ($p > 0.05$). At the same time, the digestibility of the fat has increased in the experimental groups with dry larvae ($p \leq 0.05$). Blood counts in all groups were within the physiological norm. As compared to control group, piglets of the experimental groups had an increase in bactericidal, lysozyme activity of the blood serum and phagocytic activity of neutrophils. Thus, supplementation of dry larvae of superworm or waxworm in the ration didn't affect negatively growth performance of experimental piglets while improving their parameters of non-specific immunity.

Keywords: Animal feed. Diet. Insecta. Nutrition.

1. Introduction

Insects represent a promising alternative of animal protein in the future. Their nutritional value, combined with their food conversion efficiency, low water requirements and ecological impact make them more economically prudent as a source of animal protein in the production of milk and meat (Dobermann et al. 2017; Sogari et al. 2019; Gasco et al. 2020). However, in order to find out their potential as a food source for increasing needs of the population, it is necessary to develop an infrastructure for their growth, processing, storage, distribution and marketing, as well as develop legislation for their use as a food. These steps become relevant only when we are able to breed insects in sufficient quantities to meet the potential needs for animal protein (Cortes Ortiz et al. 2016). A proven opportunity is represented in the industrial

breeding of various insect species on the diverse organic substrates (Veldkamp et al. 2012; Nekrasov et al. 2019; Antonio et al. 2020). Recently widely used Hermetia illucens is one of the examples of effective insect farming for their use in feeding of farm animals and other types of insects (Barragan-Fonseca et al. 2017; Allegretti et al. 2018; Bejaei and Cheng 2020). However, there is still not enough data on the effect of insect larvae on animal immunity.

Recent studies showed that there are several key features that make insects suitable for use in animal food: they have a high protein content (from 55% to 75% in DM); they are enriched with other nutrients such as lipids, minerals and vitamins and characterized by high food conversion rate. Therefore, they can be a very valuable source of food for farm animals (Maurer et al. 2016). It should be noted that insects are already a natural component of animal food chain as in case of carnivorous fish, poultry and pigs (for example, insects can provide up to 70% of the protein requirement in trout feeding) (Bondari and Sheppard 1987).

Currently, insect-derived proteins are not allowed in the feed designed for pigs or poultry in the European Union since they are not yet fully characterized. However, they may be used for domestic animals (such as dogs, cats, birds or reptiles) and fur animals (mink). The ban on feeding agricultural animal species with insect-derived proteins does not apply to whole insects or to fats derived from insects (Lähteenmäki-Uutela and Grmelová 2016). There are total seven permitted species that are represented by three species of crickets (*Acheta domesticus*, *Gryllodes sigillatus*, *Gryllus assimilis*), two species of flour worms (*Tenebrio molitor*, Alphitobius diaperinus) and two species of flies (*Hermetia illucens*, *Musca domestica*). The most studied to date are the yellow flour worm (*Tenebrio molitor*) (Jin et al. 2016; Yoo et al. 2019), the black soldier fly (*Hermetia illucens*) (Spranghers et al. 2018; Biasato et al. 2019) and housefly (*Musca domestica*) (Newton et al. 1977; Hussein et al. 2017). Other species for which modern methods of obtaining and using biomass were developed are the superworms (*Zophobas morio*) (Thévenot et al. 2018; Benzertiha et al. 2019) and, to a lesser extent, wax moth (*Galleria mellonella*) (Veldkamp et al. 2012). Further studies are required in order to characterize the nutritional value of these insect species for development of various alternative ration recipes for agricultural animals. Our experiment was conducted to investigate the effects of dried larvae of darkling beetles (*Zophobas morio* L.) and larvae of wax moths (*Galleria mellonella* L.) on growth performance, nutrient digestibility and blood/immunological profiles in recently weaned piglets.

2. Material and Methods

Experimental groups and treatment conditions

Crossbred ((Large White × Landrace) × Duroc) weaned pigs (39 d of age, 14.39±0.19 kg body weight) were randomly selected for control and two treatment groups (n=9 per group) based on gender and body weight. All pigs were housed at the experimental building of the L.K. Ernst Federal Research Center for Animal Husbandry. Three pigs were reared per each pen (concrete-slot floor, 1.5×2.0 m). The living conditions of all groups (humidity, light conditions and gas composition of the air in the room) were kept at the level of required health standards. The temperature was kept at 30°C during first week and then lowered 1°C every week. The experiment was conducted for 42 days. The research were conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123, Strasbourg, 1986). The research was approved by the bioethical commission of the L.K. Ernst Federal Research Center for Animal Husbandry (protocol #2019-04/1, dated Apr 04, 2019).

Feed ration and treatments

Compound feed and water were provided *ad libitum* for all groups. The control group of animals received a wheat-corn-sunflower compound feed with 8% fishmeal, whereas experimental groups were fed with a compound feed where part of fishmeal and part of whole grain were substituted with 2.5% of dried larvae of darkling beetles (*Zophobas morio* L.) or 3.0% of dried larvae of wax moths (*Galleria mellonella* L.). The percentage of substitution was calculated based on energy and protein content in dried superworm

powder (20.81 MJ/kg metabolizable energy (ME), 45.84% crude protein (CP)) and dried waxworm powder (23.32 MJ/kg (ME), 34.40% (CP)). To balance feed recipes in terms of calcium and phosphorus, DCP administration was increased from 0.4% to 0.65%. All specified by NRC (2012) nutrient requirements were met or slightly exceeded their values in the experimental rations.

Body weight and food intake were measured at the beginning and at the end of each week to calculate average daily gain (ADG), average daily food intake (ADFI) and food to gain ratio (F:G ratio).

Insect meal preparation

Superworm larvae biomass and waxworm larvae biomass were produced by LLC «NordTechSad» (Novodvinsk, Russia). Larvae were harvested on day 20 (superworm) and day 35 (waxworm) and then air-dried in an oven at 120°C for 40 min. Upon receiving the dried biomass from grower, we grinded it to the condition of powder and then analyzed in terms of energy and nutrients values. This information is presented in Table 1. Other parameters of the feed compounds are shown in Tables 2.

Table 1. Content of nutrients in the insect biomass (dry matter base).

Items	*Zophobas morio* L.	*Galleria mellonella* L.
Crude protein, %	42.10	31.12
Crude fat, %	36.52	48.83
Chitin, %	3.14	7.42
Crude ash, %	3.29	2.26
GE, MJ/kg	24.73	27.38
ME, MJ/kg	19.11	21.09
Ca, %	0.15	0.08
P, %	0.33	0.29

GE, gross energy; ME, metabolizable energy; Ca, calcium; P, phosphorus.

Table 2. The compound feed formula and chemical composition of the rations.

Ingredients (%)	Groups		
	1-control	2- *Zophobas morio* L.	3- *Galleria mellonella* L.
Wheat	36.00	35.25	34.75
Corn	31.00	31.00	31.00
Sunflower meal	13.90	13.90	13.90
Fishmeal	8.00	6.00	6.00
Superworm larvae	-	2.50	-
Waxworm larvae	-	-	3.00
Malt sprouts	3.00	3.00	3.00
Feed yeast	3.00	3.00	3.00
Sunflower oil	2.00	2.00	2.00
Premix	1.00	1.00	1.00
Limestone	0.57	0.57	0.57
L-lysine HCl	0.50	0.50	0.50
DL-methionine	0.48	0.48	0.48
DCP	0.40	0.65	0.65
Salt	0.15	0.15	0.15
Chemical composition			
ME (MJ/kg)	13.60	13.82	13.85
Moisture (%)	10.97	10.98	10.97
Crude protein (%)	19.05	18.99	19.16
Ether extract (%)	4.30	4.38	4.38
Crude ash (%)	5.10	5.07	5.08
Total lysine (%)	1.17	1.15	1.16
Total methionine (%)	1.13	0.72	0.72
Total calcium (%)	0.80	0.78	0.78
Total phosphorus (%)	0.73	0.72	0.72

DCP, dicalcium phosphate; ME, metabolizable energy; Vitamins content per kilogram of dried biomass: vitamin A, 12,500 IU; vitamin D3, 2,500 IU; vitamin E, 80 IU; vitamin H, 0.15 mg; thiamine chloride, 1.0 mg; riboflavin, 4.0 mg; calcium pantothenic acid, 25 mg; niacin, 15 mg; vitamin B12, 30 mg; vitamin K3, 1.5 mg; Mn, 30.0 mg; Cu, 160.0 mg; Fe, 100.0 mg; Zn, 110.0 mg; Co, 0.2 mg; Se, 0.3 mg; I, 1.0 mg.

Apparent total tract digestibility

Nine crossbred pigs (29.88±0.18 kg) were housed in individual metabolic crates and were randomly assigned to control and experimental groups with three replicates per group. Each pig was fed 800 g of ration twice a day at 7:00 and 18:00. After adaptation period (5 days), samples were collected for following five days. Plastic containers for urine samples contained 50 mL of 4N H_2SO_4 to prevent evaporation of nitrogen prior to retention analysis. Fecal and urinary samples were stored at −20°C until the end of experiment. The feces were dried in a drying oven at 60°C for 72 h and then ground to 1 mm size in a Laboratory mill (LMC-1M, NV-Lab, Russia) for the chemical analysis including moisture, protein, fiber and fat contents by AOAC methods (1995).

Blood sampling

All pigs selected for physiological experiments (n=9) were bled through the anterior vena cava in the end of experiment. Blood samples were collected in disposable culture tubes and centrifuged for 5 min (6000 rpm at 4°C) (centrifuge, Hettich GmbH & Co KG, Germany). The serum was carefully transferred to 1.5 mL micro tubes and stored at −20°C until analysis. Separately, seven ml of venous blood was collected in tubes with EDTA for immunological analysis and transported to the laboratory within two hours.

Chemical analysis

Compound feed and feces were grounded with a Laboratory mill (LMC-1M, NV-Lab, Russia) and then analyzed for dry matter (DM) and crude ash (CA) (ISO 6496-83 and ISO 5984 respectively). The nitrogen content was analyzed with ISO 5983-2-2016, from which crude protein (CP) content was calculated with formula (Nitrogen×6.25), crude fiber with ISO 6865-2015, crude fat ISO 6492:1999, calcium (Ca) ISO 6490-1:1985, phosphorus (P) ISO 6491-2016.

Blood serum was analyzed for calcium, phosphorus, magnesium (Mg), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), creatinine (CREA), cholesterol (CHOL), glucose (GLU), total protein (TP), albumin (ALB) and urea (UREA) using ChemWell 2910 automatic EIA and Chemistry analyzer (Awareness Technology, USA).

Immunological analysis of blood

Whole blood was analyzed for erythrocytes (RDC), leukocytes (WBC), hemoglobin (HGB), hematocrit (HCT) using ABC VET analyzer (Horiba ABZ, France) with Uni-Gem reagent kits (ReaMed, Russia).

Bacterial strains and culture conditions

Strains *E. coli ATCC 25922* and *M. luteus (lysodeicticus) ATCC 4698* were obtained from Federal Budget Institution of Science «State Research Center for Applied Microbiology & Biotechnology». These bacteria were cultured in Tryptic Soy Agar (TSA) (Merck, Germany) at 37°C for 24 h. The cultures were suspended in phosphate-buffered saline and adjusted by Densi-La-Meret (PLIVA-Lachema Diagnostika, Czech Republic) for phagocytosis assay to 4.5 McF (*E. coli*), for bactericidal activity assay to 1.9 McF (*E. coli*), for lysozyme activity assay to 0.6 McF (*M. luteus*) and used within 15 minutes.

Phagocytosis assay

E.coli culture (0.5 ml) was added to 0.5 ml of blood and incubated on shaker at 37°C for 30 min. The sediment of mixture was smeared, fixed with 96% methanol, stained with Romanowsky-Giemsa method and viewed under microscope (90×). E.coli-engulfed neutrophils were counted as positive cells. We analyzed 100 neutrophils per slide. The following parameters were determined: Phagocytic activity (PA) = (Number of neutrophils involved in phagocytosis/all neutrophils)*100%; Phagocytic index (PI) = Number of *E. coli* cells

ingested/100 active neutrophils; Phagocytic amount (PAM) = Number of phagocytosed bacteria cells/all neutrophils.

Lysozyme activity assay

Lysozyme was measured by turbidimetric method in a spectrophotometer UNICO-2100 (United products & instruments, Ins. USA) at OD540. Tubes of blood serum (0.1 ml) were heated (56ºC) in a water bath for 30 minutes, then 1.4 ml of standard *M. luteus* culture was added and incubated at 37°C for 3 h. The following parameters were determined: lysozyme activity of blood serum (LA), concentration of serum lysozyme (lysozyme, µg/mL), activity unit (AU) per 1 mg protein (AU/TP).
Lysozyme activity (LA) of a blood serum is calculated using the following formula:

$$\%LA=((\Delta Do)*100/Do1) – ((\Delta Dk)*100/Dk1)$$

Do is the difference in the optical density of the prototype,
Dk is the difference in the optical density of the control,
Do1 is the optical density of the prototype immediately,
Dk1 is the optical density of the control.

The concentration of lysozyme in serum was calculated based on calibration with dilutions of chicken egg-white lysozyme (L6876, Sigma) ranging from 0.1 to 51.2 µg/ml.

Due to variations in protein content in the blood serum of animals, the level of lysozyme activity was converted and expressed in arbitrary units of activity per 1 mg of protein (activity units per 1 mg of TP or AU/TP).

Bactericidal activity of blood serum

Bactericidal activity (BA) of blood serum was measured by turbidimetnic method in a spectrophotometer UNICO-2100 (United products & instruments, Ins. USA) at OD540. *E. coli* culture (0.005 ml) was mixed with 4.5 ml of Tryptic Soy Broth (TSB) (Merck, Germany) and 0.5 ml of blood serum in sterile tubes. Control was 0.5 ml of physiological saline with phosphate buffer instead of serum. All tubes were cultured at 37°C for 5 h.

Percentage of BA was calculated from the following formula:

$$\%BA=((Dk-Do)/Dk))*100$$

Dk is optical density of control;
Do is optical density of experimental sample.

Statistical analysis

Statistical analysis was carried out by least squares mean comparisons using PDIFF option of general linear model procedure (SAS, 2002; SAS Inst. Inc., Cary, NC, USA). Each pen was considered as an experimental unit in measuring growth performance, while individual pig was used as experimental unit for analyzing nutrient digestibility, nitrogen retention and blood characteristics.

Statistical differences were considered highly significant at $p<0.01$, significant at $p<0.05$ and as tendency between $p\geq0.05$ and $p\leq0.10$.

3. Results

The effects of supplementation of dried superworms and waxworms on growth performance in weaning pigs are presented in Table 3. The body weight, ADG and F/G ratio did not differ among groups in the end of experiment.

Addition of the dried larvae biomass to the ration showed improvement in digestibility of a crude fat by 15.3% in the second group and 14.6% in the third group ($p = 0.05$ and $p < 0.05$, respectively) (Table 4). However, there were no significant differences in the digestibility of dry matter, crude protein, crude fiber, and nitrogen retention.

Table 3. Effect of dried superworm and waxworm supplementation on growth performance in growing pigs (n=27).

Items	Group		
	1-control	2- *Zophobas morio* L.	3- *Galleria mellonella* L.
Body weight (kg)			
Initial	14.24±0.40	14.37±0.34	14.54±0.29
42 days	31.54±1.03	31.72±1.02	31.92±1.32
Average daily gain (g)	411.90±24.07	413.23±20.65	413.76±21.81
Average daily feed intake (g)	1,200	1,200	1,200
Feed:gain ratio (F/G)	3.02±0.24	2.97±0.18	3.02±0.24

Kg, kilogram; g, gram; F, feed; G, gain.

Table 4. Effect of larvae supplementation on nutrient digestibility in growing pigs (n=9).

Items	Groups		
	1-control	2 - *Zophobas morio* L.	3 - *Galleria mellonella* L.
Nutrient digestibility (%)			
Dry matter	74.34±1.58	74.88±1.37	75.04±1.06
Crude protein	75.49±3.42	74.02±1.63	77.29±1.87
Crude fiber	37.96±1.48	40.30±3.28	40.90±4.14
Crude fat	29.75±4.63	45.00±2.86*	44.36±0.70*
		N (g/d)	
N-intake	4.88	4.86	4.90
N-feces	1.09±0.13	1.10±0.07	0.97±0.08
N-urine	1.69±0.07	1.70±0.11	1.90±0.13
N-retention	2.10±0.17	2.06±0.07	2.04±0.19
		Ca (g/d)	
Ca-intake	1.280	1.248	1.248
Ca-feces	0.807±0.028	0.783±0.080	0.786±0.024
Ca-urine	0.030±0.002	0.022±0.002	0.028±0.003
Ca-retention	0.443±0.026	0.443±0.082	0.434±0.025
		P (g/d)	
P-intake	1.168	1.152	1.152
P-feces	0.385±0.018	0.384±0.041	0.397±0.018
P-urine	0.281±0.014	0.156±0.009**	0.240±0.039
P-retention	0.502±0.007	0.612±0.049	0.515±0.025

*- $p < 0.05$; **- $p < 0.01$ vs control; N, nitrogen; Ca, calcium; P, phosphorus.

Similarly, there were no significant changes in P and Ca retention in all groups. P excretion with urine was lower in the second experimental group ($p<0.01$) that slightly changed its retention toward increase ($p = 0.07$, group 2 vs. control). Decreased excretion of Ca with urine in animals of this group ($p <0.05$) did not affect its retention in the body.

The biochemical parameters of a blood serum were analyzed at the end of the experiment and are presented in Table 5. Although we did not observe significant differences between groups, the level of albumin was highest in the blood serum of the third experimental group ($p < 0.01$). As a consequence, this resulted in substantial increase in the protein index (ALB/GLB ratio) in that group ($p = 0.05$). The morphological parameters of the blood in all groups were within the physiological norm; the number of leukocytes (WBC) was significantly higher in the blood of the second group as compared to controls ($p < 0.05$).

Table 6 shows the results of the immunological study. Most of the analyzed parameters were higher in the experimental groups as compared to controls, except for BA. However, these differences were not significant statistically. Phagocytic Index (PI) was also higher in experimental groups, with a second group

expressing it more robust than third (p = 0.08). Among studied parameters, only PAM was significantly increased by 20.8% in the third group (p < 0.05).

Table 5. Biochemical and morphological parameters of the blood (n=9).

Parameters	Group		
	1-control	2- *Zophobas morio* L.	3- *Galleria mellonella* L.
TP, g/L	61.65±7.84	59.10±9.27	59.42±4.98
ALB, g/L	21.23±1.63	27.51±5.96	33.60±1.66**
GLB, g/L	40.43±8.56	31.59±3.33	25.82±1.51
ALB/GLB ratio	0.59±0.17	0.85±0.11	1.43±0.08*
UREA, mmol/L	4.17±0.49	4.16±0.43	5.04±0.64
CREA, mmol/L	94.10±4.96	81.45±7.82	84.58±15.62
TBIL, µmol/L	4.08±1.16	6.12±1.34	5.24±1.01
ALT, IE/L	49.17±0.10	48.97±0.26	44.90±8.28
AST, IE/L	51.65±0.20	53.03±1.79	48.27±4.73
ALP, mmol/L	291.0±36.81	324.02±69.55	275.27±68.91
CHOL, mmol/L	2.65±0.22	2.77±0.12	2.68±0.39
GLU, mmol/L	4.88±1.17	7.56±1.47	7.22±1.98
Ca, mmol/L	2.49±0.33	2.67±0.10	2.59±0.18
P, mmol/L	2.87±0.33	3.29±0.18	3.03±0.11
Ca/P	1.14±0.17	1.06±0.09	1.12±0.11
Mg, mmol/L	0.80±0.06	0.63±0.10	0.72±0.07
WBC,10^9/L	10.44±0.70	12.36±0.18*	12.89±1.33
RBC,10^{12}/L	10.96±0.34	11.15±0.27	10.28±0.41
HGB, g/L	115.7±4.80	127.63±3.92	120.80±5.72
HCT, %	60.52±1.26	60.61±1.40	56.63±2.82

*- p < 0.05; **- p < 0.01 vs control; TP, total protein; ALB, albumin; GLB, globulin; UREA, urea; CREA, creatinine; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; CHOL, cholesterol; GLU, glucose; Ca, calcium; P, phosphorus; Mg, magnesium; WBC, leukocytes; RDC, erythrocytes; HGB, hemoglobin; HCT, hematocrit.

Table 6. Parameters of non-specific immunological response of the blood (n=9).

Parameters	Group		
	1 -control	2 - *Zophobas morio* L.	3 - *Galleria mellonella* L.
LA, %	15.24±1.90	20.47±2.65	16.19±0.48
Lysozyme, mkg/mL	0.31±0.03	0.39±0.04	0.33±0.01
AU/TP	2.56±0.46	3.12±0.52	2.66±0.27
BA, %	65.83±2.20	60.00±2.50	60.83±2.20
PA, %	35.09±1.52	37.33±1.76	39.10±1.99
PI	2.06±0.06	2.30±0.08	2.22±0.11
PAM	0.72±0.04	0.86±0.06	0.87±0.02*

*- p < 0.05 vs control; LA, lysozyme activity; AU/TP, activity unit (AU) per 1 mg total protein (TP); BA, bactericidal activity; PA, phagocytic activity; PI, phagocytic index; PAM, phagocytic amount.

4. Discussion

Studies that provide feedback on the use of insect biomass as a substitute for a part of protein content in the feed are receiving increased attention worldwide. More studies will ensure the possibility of entering insect products into compound feeds for animal farming. Per this growing interest, we focus our study on evaluation of minimal substitution of fishmeal with insect biomass on young pigs.

Our research showed that mild replacement of fishmeal by superworms or waxworms (2%) in the ration did not change feed intake, feed conversion and growth parameters of the pigs. In this regard, our data are similar in outcome with studies by other authors (Ji et al. 2016; Chia et al. 2019). Crude fat digestibility was increased in experimental groups (p <0.05, control vs 2 & 3 groups), which is similar to the result of the study conducted by Jin et al. (2016) using dried mealworm (1.5 to 6%) in weaning pigs. Increase in digestibility of the fat that we observed could be associated with the fatty acid composition of lipids in insect larvae. According to Kierończyk et al. (2018), insect fats have higher concentrations of monounsaturated and lower polyunsaturated fatty acids as compared to other fats. Despite of high saturated fatty acid content of insect-based diets, the digestibility rates of all fatty acids are high. In regards

to fat digestion, Zhang et al. (2018) reported that inclusion of insect larvae in the diet of growing pigs resulted in elevated high-density lipoprotein concentration in the serum. Interestingly, although we did not analyzed high-density lipoprotein (HDL) concentration, we noted slightly higher cholesterol level in experimental groups. In normal physiological conditions, cholesterol binds to HLD for excretion in the liver. Thus, slight increase in cholesterol in our experimental groups could indicate counteraction to HLD level. Further more focused studies can help to clarify if there is significant impact of insect diet on lipid methabolism and how long exposure to this biomass could effect health and outcomes (Belghit et al. 2019).

Analysis of hematological and biochemical parameters of the blood is considered a standard procedure for assessment of the nutritional and health status of animals (Biasato et al. 2019). It was noted previously that partial replacement of fishmeal with dried insect larvae did not adversely affect the biochemical parameters of blood serum (Maurer et al. 2016). This was also observed in our experimental pigs. Most of the parameters were similar to control animals or changed insignificantly, except for ALB concentration. TP and ALB concentrations in the blood serum indirectly characterize protein digestion as well as amino acid (AA) absorption and metabolism. Albumin is the most favorable source of amino acids for protein synthesis. In our study, pigs that received dried insect larvae had TP values comparable to control, while serum ALB was higher ($p < 0.05$, group 3 vs control). This result may indicate that pigs fed with the dried larvae utilized this protein source more optimally as indicated by ALB/GLB ratio. The lower ratio, the better use of protein and opposite. The lower excretion of phosphorus in experimental pigs of the third group indicates its better utilization or its more robust retention in the organism of animals receiving insect biomass. It is known that the digestibility of phosphorus in plant-based feeds is often low. Based on our data and research by Poulsen (2000), it is necessary to reevalute the supplemention of rations with additional phosphorus when the insect biomass is added.

Despite economically beneficial perspective, substitution with insect biomass requires careful consideration, because monogastric animals such as pigs and poultry are very sensitive to contaminated food (Chia et al. 2019). It is known that insect larvae can accumulate various types of toxins, heavy metals, and other xenobiotics (Purschke et al. 2017). However other researchers confirmed that mycotoxins are not found in larvae even when grown on a contaminated substrate (van Broekhoven et al. 2014). Only few cases of allergic reactions after consuming silkworm pupae, cicadas and crickets have been reported in China (Feng et al. 2017). As per our study, we did not notice any obvious adverse toxic effects of experimental ration through studied parameters.

On the contrary, there is evidence of improved immune response in animals that received insect larvae in the literature (Benzertiha et al. 2019). In our study, we looked at humoral and cellular factors of the immune defense including lysozyme, bactericidal and phagocytic blood activity. The use of insect larvae slightly improved protective immune properties in our experimental pigs, which is consistent with data obtained by other researchers (Renwick et al. 2007; Veldkamp et al. 2012; Benzertiha et al. 2019). The increased level of WBC correlated with an increase in the phagocytic activity of the blood (PA, PI, PAM). The effect on immune status in our study could be related to the amount of insect substitute in the feed. Bioactive components such as lauric acid, antimicrobial peptides, chitin and larval fat containing large amounts of medium-chain fatty acids with antimicrobial properties could influence the response to infectious pathogens (Schiavone et al. 2017, 2018; Secci et al. 2018; Iaconisi et al. 2017; Hussein et al. 2017, Allegretti et al. 2018; Spranghers et al. 2018). These components are known to stimulate both cellular and humoral defense mechanisms against intracellular and extracellular pathogens (Mak et al. 2010; Wojda 2017; Cutuli et al. 2019; Chia et al. 2019). We suggest that the immune status of growing pigs can be regulated by adjusting the level of insect biomass in their feed.

5. Conclusions

The direction of the study is justified and relevant in connection with the shortage of animal feed. New data on the effectiveness of larvae biomass as substitute for fishmeal in the feed of various types of farm animals allows us to consider it as promising.

Inclusion of dried larvae (*Zophobas morio* and *Galleria mellonella*) at 2% instead of full dose of fishmeal is acceptable practice for retaining the growth performance. Dried larvae supplementation does not reduce feed intake and nutrient digestibility and does not detrimentally affect immune response.

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