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Effects of replacing soybean oil with selected insect fats on broilers

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Highlights

- The fatty acid profiles of *Tenebrio molitor* and *Zophobas morio* were characterized.
- No negative effects of insect oils on growth performance were recorded.
- The use of *T. molitor* oil positively affected the digestibility of nutrients.

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- Insect oils caused some up- and downregulation of gene expression in the liver.
- Insect oils affected the fatty acid composition of liver and breast tissue.

Abstract

The aim of this study was to investigate how oil obtained via super-critical CO_2 extraction from *Tenebrio molitor* (TM) and *Zophobas morio* (ZM) larvae affect the growth performance, nutrient digestibility, lipid fatty acid composition of liver and breast tissue, and the expression of selected genes in the liver of broiler chickens. Two independent experiments were conducted on 72 and 108 one-day-old female Ross 308 chicks, respectively. Birds were fed soybean-maize diets developed by replacing 50 g/kg of the basal diet with various fats i.e., soybean oil (SO) and TM (Exp. 1), or SO, TM, and ZM (Exp. 2). In both trials, birds were kept in metabolic cages over a 28 day period. The fatty acid profile of used energy sources was determined. Both insect oils had higher monounsaturated fatty acids (MUFA) and lower polyunsaturated fatty acids (PUFA) concentrations comparing to SO. The addition of TM and ZM oil to the basal diet showed similar or better growth performance results compared to the SO diet over the entire experimental period. Insects' oils addition increased nitrogen retention and the apparent digestibility of the ether extract in the total tract at days 7, 14, and 21 during TM administration. Similar effects on the apparent ileal digestibility of crude protein and ether extract or on AME_N were recorded among all groups in the second trial. The usage of selected insect oils significantly affected the fatty acid compositions of liver and breast tissue. Only TM addition had a positive effect on the PUFA ($P=0.004$), MUFA ($P<0.001$), UFA ($P=0.016$), and SFA ($P=0.016$) of breast muscle. Simultaneously, the TM treatment lowered the thrombogenic ($P=0.011$) and atherogenic ($P=0.001$) indices in the breast. The positive influence of insect oils addition to the basal diet on selected gene (*HNF4a; APOA1; GIMAP5*) expression in the liver was observed. Overall, these results highlight the possibility of completely replacing SO with TM and ZM oils in broiler diets without adverse influences on growth performance and nutrient digestibility. Moreover, the results of the present study suggest that TM oil positively affect meat quality which is a key factor for the modern consumer. It should be emphasized that both insect oils used in the study may be considered as a biologically active compounds modify molecular pattern at the mRNA level.

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Keywords: broiler chickens; *Tenebrio molitor*; *Zophobas morio*; dietary fat; performance; digestibility.

Introduction

Insects are a potential protein source in poultry (Bovera et al., 2016; Józefiak et al., 2016; Maurer et al., 2016), swine (Jin et al., 2016), fish (Henry et al., 2015) and companion animal nutrition (Bosch et al., 2014). The crude protein (CP) content of insects is species-dependent and varies from 40% to 60% (Makkar et al., 2014). It should be emphasized that the CP quality also depends on the production technology and feed composition for larval rearing (Tschirner and Simon, 2015). However so far, in the European Union, the usage of insect meal in livestock nutrition is banned (Regulation (EC) No. 1069/2009) because these compounds are considered to be processed animal protein (PAP, Regulation (EC) No. 999/2001). Currently, the EU Standing Committee on Plants, Animal, Food and Feed (SCoPaFF) only allows the use of insects as a protein source in the case of fish, mink and pet-food nutrition. In addition to protein, edible invertebrates at all life stages are rich sources of other valuable nutrients, including crude fat. This raw material may be an alternative to replace resource-intensive and more expensive soybean oil, palm kernel oil, coconut oil, and fish oil. Additionally, from a legislative point of view, insect fat can be used in poultry diets in the EU. The authors estimate that in Poland alone, 240,000 tonnes of dietary fat is used for broiler chicken nutrition annually, at a total cost of more than 170 M USD. Therefore, there is a large market for novel sources of dietary fats, including insect-derived ones, and an emphasis should be placed on the yield and quality of the insect fat. Up to date, there is limited data about the usage of insects as an alternative energy source for poultry, including broiler chickens. Only few insect species were taken into consideration in animal nutrition, however, the emphasis is put on *Hermeria illucens* larvae, in this case (Schiavone et al., 2016). Whereas, there is a wide spectrum of edible insect species available on the market. In

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the present study, *Tenebrio molitor*, as well as *Zophobas morio*, were chosen as an alternative energy source in view of the fact that are commonly available, their production is seamless and well understood, as well as their fat content is relatively high. For instance, in most Tenebrionidae larvae, fat can constitute more than 30% of the dry matter content. In the case of *T. molitor*, it can reach up to 43% crude fat (Józefiak et al., 2016). Furthermore, the quality of insects' fat is comparable to already used energy sources in animal production. As DeFoliart (1991) reported, the degree of unsaturated insect fatty acids is similar to fish oil; however, insect fatty acids are richer in polyunsaturated fatty acids (PUFA). In general, the dominant fatty acids of insects are oleic, linoleic (LA) and palmitic acids (Jones et al., 1972; Martin et al., 1976; Finke, 2002; 2013). However, Bukkens (1997) suggested that the fatty acid profiles are species-dependent and reflect insects' feed composition.

Currently, the soybean oil is one of the most commonly used energy source ingredient in the poultry diets, due to its high metabolizable energy content, as well as digestibility. Whereas, the price of this compound increase annually, and the supply of non-genetically modified soybeans is limited. From the above-mentioned reasons, it is crucial to expand knowledge about replacing soybean oil by alternative sources such as insects' origin fat. Hitherto, the experiments carried out on *H. illucens* fat suggests no adverse effects on the growth performance, carcass traits, as well as overall meat quality (Schiavone et al., 2018). However, in the available literature, there is a lack of data on *T. molitor* and *Z. morio* as novel fat sources for poultry. Therefore, the aim of this study was to examine how fats obtained from *Tenebrio molitor* and *Zophobas morio* using super-critical CO_2 extraction affect the broiler chicken growth performance, nutrient digestibility, lipid fatty acid composition of liver and breast muscle tissue, and expression of selected genes in the liver.

Materials and Methods

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Diets

The composition of the basal diet is shown in Table 1. In both experiments, birds had *ad libitum* access to water and feed for 28 days. The composition of basal diets was designed according to Tancharoenrat et al. (2013) and was formulated on the basis of maize and soybean meal. The basal diet were developed by substituting soybean oil (SO), *T. molitor* oil (TM; Exp. 1) or SO, TM, and *Z. morio* (ZM; Exp.2) dietary fats for 50 g/kg of the basal diet. The crumbled form of the diets was produced in the Piast Pasze factory (Lewkowiec, Poland) according to ISO 9001:2008 procedures. The diets did not contain any feed additives, such as ionophore coccidiostats, exogenous enzymes, and so on. In the entire experiment, 0.2% of titanium dioxide (TiO_2) was added as an internal marker for calculation of nutrient digestibility.

Insect fat composition

The three fat sources (SO, TM, and ZM) were obtained from commercial sources (HiProMine S.A, Poland). The fatty acid composition of these fats is presented in Table 2. Dietary insect fat was obtained using super-critical CO_2 extraction according to Jackowski et al. (2015). Briefly, before the extraction, materials were air-dried in an oven (SLN 240, POL-EKO Aparatura, Poland) for 24 h at 50°C. After that, the following parameters were used for the extraction: pressure – 300 bar, temperature – 40°C. For extract collecting the two-separator system was used. The CO_2 flow was adjusted to 110.4 kg/h. Commercial CO_2 (99% purity, Zakłady Azotowe, Puławy, Poland) was used for the extraction.

Animal and sample collection

All procedures and experiments complied with guidelines and were approved by the Local Ethics Commission of the Poznań University of Life Sciences (Poznań, Poland) with respect

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to animal experimentation and care of the animals being studied. All efforts were made to minimize suffering.

Experiment 1

A total of 72 one-day-old female Ross 308 chicks obtained from a commercial hatchery were randomly assigned to 2 dietary treatments. Each treatment had 12 replicates and 3 birds per replicate. Birds were kept in metabolic cages (40 x 40 x 40 cm) over a 28 day period. Birds were given 23 h of light and 1 h of dark during the first week and then 19 h of light and 5 h of dark from day 7 to day 21. From 22 to 28 days of age, they were provided 23 h of light and 1 h of dark. The temperature was maintained at 32°C on day 1 and was gradually reduced to 21°C by day 21 and maintained. Birds were weighed on days 1, 7, 14, 21, and 28. The body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were calculated for days 1-7, 7-14, 14-21, 21-28, 1−28. Samples of fresh excreta from each replication tray (n = 12) were collected on days 7, 14, 21, and 28 to measure nitrogen retention, as well as analysis of apparent total tract digestibility of ether extract. Excreta sample pooled within a cage represented one replication (3 birds). Samples were immediately frozen, freeze-dried, grounded, and stored at - 20°C for future analysis (Ptak et al., 2013).

Experiment 2

A total of 108 one-day-old female Ross 308 chicks were randomly assigned to 3 dietary treatments. Each treatment had 12 replicates and 3 birds per replicate. Birds were kept in metabolic cages (40 x 40 x 40 cm) over 28 days. The environmental conditions (e.g., temperature, light program) were the same as in the first experiment. The growth performance was also measured as described in the above section. At the end of experiment (day 28), all chickens were killed by cervical dislocation. During dissection, the digesta from the ileum

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were gently squeezed by segments from 12 individual birds (randomly chosen) per treatment for further apparent ileal digestibility analyses. The ileum was defined as the small intestinal segment caudal to Meckel's diverticulum. Immediately after slaughter, the livers were cut into small pieces (0.4 cm x 0.4 cm) and deposited in approximately 0.7 mL of fix RNA (E0280, EURx, Poland) for protection from degradation prior to RNA isolation.
Simultaneously, breast muscle and liver tissue were cut, directly packaged in flexigrip bags and put into dry ice for further analyses.

*RNA isolation and reverse transcription quantitative PCR (*RT-qPCR*) reaction*
Liver tissues were homogenized with TissueRuptor homogenizer (Qiagen GmbH, Hilden, Germany) in TRIzol® LS Reagent (Ambion/Thermo Fisher Scientific, Valtham, USA). Further steps of RNA isolation were performed with a commercial kit (Universal RNA Purification Kit, EURx, Gdańsk, Poland). RNA quality and quantity was verified by electrophoresis on 2% agarose gel and NanoDrop 2000 (Scientific Nanodrop Products, Wilmington, USA). cDNA was synthesized using a Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific/Fermentas, Vilnius, Lithuania), following the manufacturer's recommendations. The obtained cDNA was diluted to 70 ng μl^{-1} and stored at -20°C. RT-qPCR reactions were conducted with a total volume of 10 µL. The reaction mixture consisted of Maxima SYBR Green qPCR Master Mix (Thermo Scientific/Fermentas, Vilnius, Lithuania), 1 µM of each primer, and 2 µl of diluted cDNA (140 ng). Thermal cycling was performed in a LightCycler II 480 (Roche Diagnostics, Basel, Switzerland). Each RT-qPCR reaction was conducted in two technical replicates. Gene expression analysis was performed for a selected panel of genes related to cholesterol level regulation (*APOA1*, *CETP*, *ABCG8*), lipid metabolism (*ACOX2*, *IRS2*, *ANGPTL4*, *HNF4A*), energy metabolism (*PPARGC1A*), protein metabolism (*UPS18*), and the immune system (*GIMAP5*). Sequences

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of primers were based on the literature (Sevane et al., 2014) or designed based on cDNA nucleotide sequences using NCBI Primer Blast.

Relative quantification of gene expression

Relative gene expression analysis was conducted separately for each experimental group using the ΔΔCt method with *UB* and *G6PD* as reference genes. Geometric means of Ct (cycle threshold) values of reference genes were used in the analysis. For each of the samples, the Ct differences between target and reference genes were calculated. Control (C) samples were used as calibrators. ΔΔCt was calculated by deducting the ΔCt value of the calibrator from ΔCt of the unknown sample. For the calculation of the normalized expression level of the gene, the following formula was used: $R = 2^{-\Delta\Delta Ct}$.

Chemical analyses

The dry matter (DM) and crude fat (CF) content of diets, digesta and excreta were determined according to AOAC (2005) using 934.01 and 920.39 methods, respectively. For titanium dioxide (TiO_2) analysis, the samples were prepared in accordance with Myers et al. (2004) and the concentration was estimated using the procedure described by Short et al. (1996). Gross energy (GE) was determined using an adiabatic bomb calorimeter (KL 12Mn, Precyzja-Bit PPHU, Poland) standardized with benzoic acid.

Lipids from the liver and breast tissues were saponificated using the procedure described by Głogowski et al. (2010). Briefly, the liver or breast muscle tissues were homogenized and placed in a screw-cap Tefl on-stoppered tubes (Pyrex, 15 mL). The mixture of 1 mL 2 M KOH in water and 1 mL 1 M KOH in methanol was added. The samples were heated up to 95°C for 10 min and cooled at room temperature for 10 min. After that samples were sonicated for 10 min. The prepared mixture was protected from the light and kept overnight in

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a screw-cap Tefl on stoppered tubes under nitrogen at 23°C. Afterwards, the obtained solution was vortexed and acidified by 4 M HCI to lower pH below 2. The extraction procedure was performed four times using diethyl ether. The extracted fatty acids were esterified using 0.5 M NaOH in methanol and subsequently converted to fatty acid methyl esters using borontrifluoride (Fluka). Fatty acid methyl esters in the SO, TM, and ZM, as well as in breast muscle and liver tissues, were determined by gas chromatography according to Cieślak et al. (2013). Briefly, a gas chromatograph (GC Bruker 456-GC, USA) fitted with a flame ionization detector (FID) and a 100 m fused-silica capillary column (0.25 mm i.d.) coated with 0.25 µm Agilent HP (Chrompack CP7420) was used. Hydrogen was used as the carrier gas at a flow rate 1.3 mL/min. Injector and detector temperatures were 200°C and 250°C, respectively. The oven temperature was programmed as follows: initially 120°C for 7 min, then increased by 7°C/min to 140°C, held for 10 min and then increased by 4°C/min to 240°C. One microliter sample volume was injected into the column. Fatty acids were identified based on their retention times and were expressed as the proportion of the sum of identified fatty acids (g/100 g FA). Observed peaks were identified by the comparison of retention times with appropriate fatty acid methyl ester standards (37 FAME Mix, Supelco, Poole, UK), using Galaxie Work Station 10.1 (Varian, CA, USA).

Calculations

The apparent total tract and ileal digestibility of crude protein and ether extracts, as well as nitrogen retention, were calculated relative to the ratio of titanium dioxide (dietary marker) to determine the nutrient content in feed or excreta/digesta according to Kaczmarek et al (2016). The following equation was used (crude protein digestibility calculation as an example):

$$D_{\text{crude protein}} = 1 - \left[\left(\frac{\text{TiO}_2 \frac{\text{g}}{\text{kg diet}}}{\text{TiO}_2 \frac{\text{g}}{\text{kg}} \text{digesta or excreta}}\right) x \left(\frac{\text{Crude protein} \frac{\text{g}}{\text{kg}} \text{digesta or excreta}}{\text{Crude protein} \frac{\text{g}}{\text{kg}} \text{diet}}\right)\right]$$

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Statistical analysis

The experiments had a completely randomized design. All data were tested for normal distributions using the Kolmogorow-Smirnov test. An analysis of variance was conducted using Bartlett's test. The significance of differences among groups was determined with the Duncan's multiple range test at the significance level of $P<0.05$. For the relative quantification of gene expression, statistical analyses were performed by comparing the Ct value of each experimental group with that of the control group with a Student's *t*-test ($P<0.05$). The analyses were performed using SAS software (version 5.0, Iowa, USA). In both experiments the following general model was used:

$$Y_i = \mu + \alpha_i + \delta_{ij}$$

where Y_i is the observed dependent variable, μ is the overall mean, α_i is the effect of dietary fat source, and δ_{ij} is the random error.

Results

Fatty acid profile in the diet

Experiment 1 and 2

The fatty acid profiles of the selected fat sources are summarized in Table 2. In each fat source, the unsaturated fatty acids (UFA)were dominant. The highest amount of UFA, mainly oleic and linoleic acid, was present in SO (84.0%), followed by TM oil (78.6%), and the lowest value was observed in ZM oil (57.8%). Furthermore, insect oils had a higher concentration of MUFA in comparison to SO, and SO had more PUFA (both linoleic and linolenic) than the other oils used in this study. The fat obtained from *Z. morio* was characterized by the highest concentration of SFA (42.2%). Additionally, the TM oil and SO had lower values of SFA, 21.4% and 16.0%, respectively. The main SFAs were palmitic acid

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and stearic acid. In the case of n-6 and n-3 fatty acids, both insect oils contained lower values relative to SO. However, ZM had the highest n6:n3 ratio.

Growth performance

Experiment 1

The growth performance results are summarized in Table 3. In the first period, no significant differences among the treatments were recorded. A positive effect of TM was observed from days 7 to 14 as BWG ($P<0.001$) and FCR ($P<0.001$) improvement. Whereas FI ($P<0.001$) was lowered by insect oil inclusion to the broiler diets. In the next period, from days 14 to 21, a similar influence of insect oil was recorded, except that BWG ($P=0.545$) did not differ among groups. From days 21 to 28, no changes in growth performance were observed among treatments. In the entire experiment, TM usage caused a decrease in FI ($P=0.013$) and FCR ($P=0.034$), though BWG was not affected ($P=0.991$).

Experiment 2

The usage of insect oils did not affect the growth performance parameters in the second experiment lasted 28 days (Table 4). Only in the day 14-21 and day 21-28 periods were significant differences in BWG were observed among treatments. The highest BWG in the TM group was observed from day 14-21, while the ZM supplementation significantly lowered this value ($P=0.041$). However, in the next experimental period (days 21-28), dietary supplementation of ZM significantly improved BWG compared to the control (SO) treatment. Both TM and ZM increased the FCR value ($P=0.048$) until day 7. From days 14 to 21, the highest FCR value was observed in the ZM group.

Digestibility

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Experiment 1

The apparent digestibility of crude fat in the total tract, as well as the nitrogen retention, are shown in Table 5. Adding TM to the basal diet increased nitrogen retention at day 7 ($P=0.017$), day 14 ($P<0.001$), and day 21 ($P<0.001$) in comparison to the soybean oil treatment. The highest value was observed on day 7 where insect oil improved nitrogen retention up to 5%. Moreover, in the insect oil group, an improvement in crude fat digestibility was observed throughout the whole experiment. The addition of TM increased fat digestibility by 3% and 9%, on day 28 and day 7, respectively.

Experiment 2

There were no significant differences among treatments in the scope of apparent ileal digestibility of crude protein ($P=0.721$), ether extract ($P=0.515$), or apparent metabolizable energy corrected to zero nitrogen balance ($P=0.780$) (Table 6).

Fatty acid profiles in the liver and breast muscle

Experiment 2

The effects of insect oils on total fat content and fatty acid profiles in liver tissue are presented in Tables 7 and 8 and in breast meat in Tables 9 and 10. Statistically significant differences ($P=0.048$) in liver total fat content (g/100 g) between ZM and SO treatments were found. ZM had higher total liver fat content. Insect oil supplementation significantly affected only a few particular fatty acids in liver tissue. Significant changes were found in some saturated fatty acids, such as C16:0 ($P=0.004$), C17:0 ($P=0.017$), C18:0 ($P=0.001$), and C22:0; ($P=0.001$) in some unsaturated fatty acids, such as C18:1 *c*9 ($P=0.001$), C18:1 *c*11 ($P=0.001$), C18:2 *c*9*c*12 ($P<0.001$) and C18:3 *c*9*c*12*c*15 ($P<0.001$); and in some long-chain polyunsaturated n-3 and n-6 fatty acids. Nevertheless, in liver tissue, both selected insect oils

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tended to increase UFA levels significantly (P<0.001), mainly as an effect of increased MUFA concentration (P<0.001). Both insect oil-fed groups (TM and ZM) had significantly higher values of MUFA (P<0.001) relative to the soybean oil group. Between insect oil-fed birds, the TM group had higher values of PUFA (P<0.001), as well as n-6 (P<0.001), fatty acids. Values for both groups were lower than in the soybean oil group. The n-3 value in the liver tissue of insect oil groups was lower than SO treatment, as well. The concentrations of n-6 and n-3 in the insect oil groups resulted in significantly higher n-6 to n-3 ratios (P<0.001) in comparison to the soybean oil group. Insect oil supplementation significantly decreased SFA concentration in the TM and ZM groups.

As a result of changes in the liver fatty acid profile, the values of some desaturation indices were significantly modified (Table 8). Insect oil supplementation increased Δ9 18:1/C18:0 and MUFA/SFA indices (P<0.001), but decreased Δ5, Δ6 and Δ4 indices (P<0.001). No effects of tested oils on the artherogenic index were found, but the thrombogenic index increased (P<0.001).

The fatty acid composition of breast muscle is shown in Tables 9 and 10. The tendency (P=0.058) of both insect oils to increasing total fat content in the breast muscle was observed. Moreover, significant differences were observed in the values of C18:3 *c*9*c*12*c*15 (P<0.001), C20:4 (P=0.01), and C22:6 (P=0.001) in both insect oil supplementation groups. The TM oil addition resulted in the lowest concentration of 16:0 fatty acid (P<0.001) and the highest of C18:3 n-6 (P=0.024). Simultaneously, ZM oil lowered values of C18:2 *c*9*c*12 (P=0.021), C22:0 (P=0.036), as well as C24:1 (P=0.015). Supplementation of insect oils affected the fatty acid profile of breast tissue by increasing the value of C18:1 *c*9 (P<0.001). However, only the ZM oil group was characterized by the highest values of C16:0 (P<0.001), C16:1 (P=0.004), and C23:0 (P=0.001). These results show that the addition of ZM oil to the broiler chicken diets decreased PUFA (P=0.004), n-6 (P=0.017), n-3 (P=0.001), PUFA n-6 and n-3 (P=0.014;

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P=0.01), PUFA/SFA (P=0.037), LNA/LA (P=0.001), LCFA (P<0.001), and Δ4 (22:6 n-3/22:5 n-3) (P<0.001). The highest values of UFA (P=0.016), n-6/n-3 ratio (P=0.012), as well as Δ9 (MUFA/SFA) (P=0.006) were observed in the TM group. The ZM treatment increased MCFA (P<0.001), Δ9 (16:1/16:0) (P=0.025), 20:4 n-6/18:3 n-6 (P=0.023), as well as the thrombogenic index (P=0.011) and atherogenic index (P=0.001). The addition of both insect oils increased MUFA (P<0.001) and total C18:1 (P<0.001) in the breast muscle.

Relative gene expression

Experiment 2

The results of the relative gene expression analysis in liver tissues are presented in Figure 1. Three genes (*APOA1*, *HNF4A*, *GIMPA5*) out of the 10 genes analyzed showed statistically significant changes in expression upon oil supplementation in the chicken diet. The *APOA1* gene was upregulated in the experimental group that received ZM. The *HNF4A* gene was downregulated in the experimental group supplemented with TM. The *GIMPA5* gene was significantly downregulated in the liver tissue obtained from both experimental groups.

Discussion

Hitherto, edible insects were not considered to be an energy source for livestock nutrition, including broiler chickens. This is perplexing because the most frequent invertebrate species used as feed contain high fat concentrations, often more than 30% of the dry matter concentration. Sosa and Fogliano (2017) compared animal fats (butter, lard, beef tallow) with insect oils (superworm, yellow mealworm, lesser mealworm, cricket, cockroach) and vegetable oils (coiza, linseed, rapeseed, sesame seed, sunflower seed, pumpkin seed, rape low erucic, soybean). As a result of their analyses, it was concluded that insect oils are located between vegetable oils and animal origin fats considering their fatty acid profiles which

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showed higher SFA contents (mainly C16:0 and C18:0), as well as higher concentrations of MUFA and PUFA. The results of the present studies confirmed above-mentioned data. The fat content in the selected insect species was determined on the level of 30.8% and 33.6% for *T. molitor* and *Z. morio*, respectively. Furthermore, extracted insect oils are a rich source of mostly UFA (at least 57.8% in ZM and 78.4% in TM). The results of this study are in agreement with DeFoliart (1991), Finke (2002), Makkar et al. (2014), as well as Sánchez-Muros et al. (2014), on the scope of fatty acid profiles in TM and ZM. It should be mentioned that the high level of UFA in the insect oils used in trials might be caused by a specific type of feed or growing medium made using various fruits and vegetables (plant waste).

Various techniques of fat extraction may cause differences in yield and quality of dietary fat obtained from insects. Hitherto, the methods used for insect fat extraction in experimental conditions were cold extraction techniques, aqueous techniques, Soxhlet and Folh extractions, as well as the mechanical pressing (Kroeckel et al., 2012). It is well-documented that dietary fat efficiency, as well as nutritional value, depends on the extraction technique. Based on the author's experience, super-critical CO_2 extraction is one of the most effective methods, but not the cheapest, to obtain crude fat from insect biomass. It is in agreement with the results obtained by Purschke et al., (2017) where the super-critical CO_2 extraction was efficiently used to defatting *T. molitor*. This method is commonly used in the food industry, for instance, to extract flavor from herbs, to extract aromas from juices, and for fat, oil or cholesterol extraction and fractionation (Raventós et al., 2002). Currently, the defatting process was considered as a crude protein yield improvement in the insect meal or biofuel production (Fasakin et al., 2003; Manzano-Agugliaro et al., 2012). However, due to the EU legislation currently prohibiting the use of insect protein in poultry production, the fat fraction may be a novel, sustainable and cheaper alternative to commonly used energy sources such as soybean oil, rapeseed oil, palm kernel fatty acid distillers or fish oil.

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This study suggests that the use of insect oils, such as TM and ZM, obtained by super-critical CO_2 extraction may fully replace soybean oil in broiler chicken nutrition without any negative impact on their growth performance and nutrient digestibility. In the available literature, there is no information on the influence of the above-mentioned insect species-derived fats on livestock productivity or the quality of the products. Only few papers about *Hermetia illucens* oil usage in broiler nutrition have already been published. The results of Schiavone et al. (2016) confirmed that partial or full replacement of soybean oil with insect oil does not affect broiler growth performance, and has no adverse impact on bird health. Interestingly, a free-choice test resulted in a lack of preferences between control (soybean) or *H. illucens* oils. This is in agreement with our study where no negative effects of insect oil on feed intake was recorded. As Józefiak et al. (2014) shown, the dietary fat type and the fatty acid composition, especially in the case of fats of animal origin, may have a significant impact on the growth performance results. Unsaturated vegetable oil used in broiler chicken diets caused lower final BWG relative to animal fat in multiple studies, especially in terms of soybean oil vs. pig lard and rapeseed oil vs. beef tallow. Furthermore, results of Józefiak et al. (2014) suggest that soybean oil decreased FI in comparison to palm kernel fatty acid distillers, rendered pork fat and lard. In the present trial, no significant differences in BWG, FI, or FCR were observed (Exp. 2). However, the effect of TM supplementation (Exp. 1) on the reduction of FI ($P=0.013$) and FCR ($P=0.034$) without a negative impact on BWG ($P=0.991$), was observed. It is well-documented that a high concentration of oleic acid and a low value of linoleic acid tend to decrease FI in poultry (Newman et al., 2002). In the current study, the opposite effect was observed. However, a similar concentration of the above-mentioned fatty acids was recorded but the growth performance results were not affected (Exp. 2) or favorable for selected insect oils (Exp. 1). The data collected by Józefiak et al. (2014) also showed no changes between oils from animal or plant origins, confirming our results. Additionally, there

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is evidence that animal-origin fats are characterized by lower digestibility relative to vegetables oils, particularly in young birds (Dei et al., 2006). It is well-documented that the absorption of fat in the GIT is more efficient when it contains a higher amount of PUFAs than SFAs. It must be emphasized that the digestibility level of nutrients in this study was very high in the control diet and treatment groups from the beginning of the trials (Tables 5 and 6). This indicates the high effectiveness of using insect oils in broiler chicken nutrition, especially in the first week of their rearing. Though the metabolizable energy (EM) of each oil used in the study was not determined, the differences between the EM of feed developed by substituting SO, TM or ZM were only marginal. This suggests that the EM of insect oils is approximately the same as the soybean oil. This confirms the usefulness of insect oils as an energy source for poultry nutrition.

There are no data on the effect of edible oil supplementation on liver function. The liver is active in oxidizing triglycerides to produce energy, breaking down fatty acids and exporting large quantities of acetoacetate into blood where it can be picked up and readily metabolized by other tissues. The liver is also the major site for converting excess carbohydrates and proteins into fatty acids and triglyceride, which are then exported and stored in adipose tissue. When analyzing the fatty acid content of chicken liver, Cieślik et al. (2011) stated that the predominant saturated fatty acid (SFA) was palmitate (C16:0), followed by stearate (C18:0). Oleate (C18:1) was the most prevalent monounsaturated fatty acid (MUFA) in all samples, followed by palmitooleate (16:1). N-3 fatty acids in chicken liver were comparatively lower than SFA and MUFA. The predominant PUFA was linoleate (C18:2). Arachidonate (C20:4) was the second most important n-6 fatty acid. In the work of Majewska et al. (2016), the concentration of SFA in chicken liver amounted to 45.58%. Among saturated fatty acids, C16:0 and C18:0 occurred in the highest amount. The prevailing monounsaturated fatty acids were oleic acid (C18:1 n-9) and palmitooleic (C16:1 n-7) with average contents of 15.91%

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and 5.89% of the total composition, respectively. The n-6/n-3 ratio was 20.7:1. The results of this study suggest that both selected insect oils tended to increase the liver UFA level significantly, mainly as an effect of increased MUFA concentrations. A positive effect of using insect oils in broiler feeding was also observed in SFA concentrations. Significant decreases in SFA from 53% in the soybean oil group to 49% and 48% in the TM and ZM groups, respectively, were observed. Regardless of the type of oil fed, the following fatty acids were predominant in chicken liver tissue: saturated C16:0, C18:0, C22:0 and unsaturated C18:1, C18:2. Unfortunately, the profile of particular fatty acids led to an undesirable increase in the n-6/n-3 fatty acid ratio, as well as undesirable changes in the values of desaturation indices in insect oils. Apart from the fatty acid profile, the content of fat in the liver tissue was increasing by the ZM addition (P=0.048). These were considered to be negative qualitative changes since in European countries, chicken livers are most frequently destined for retail for direct human consumption. The limited data describing insect oils as a component of broiler diets does not allow for discussing our findings in great detail.

Recent studies have shown that the quality of chicken meat can be modified through the dietary (Aziza et al., 2010). It was found that PUFAs in tissues can especially be affected by dietary concentrations (Parveen et al., 2016). Moreover, fatty acids in different tissues can also originate from *de novo* synthesis or the bioconversion of C18:3 n-3 and C18:2 n-6 to corresponding long-chain polyunsaturated fatty acids (Masek et al., 2014). Including TM in chicken diets resulted in increased oleic acid deposition and decreased α-linolenic acid occurrence in the breast muscle. When the ZM oil was fed, the deposition of palmitic and oleic acids in the tissue increased, while the amounts of linoleic and α-linolenic acids decreased. It was expected that the inclusion of insect oil greatly affects the fatty acid profile of the breast. As Schiavone et al. (2016) showed, *H. illucens* oil supplemented to the broiler

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diet increased SFA and lowered the PUFA fraction in the breast muscle. However, *H. illucens* oil did not affect MUFA content. In this study, supplementation of both insect oils enhanced MUFAs in the breast. Additionally, the TM treatment had a higher value of PUFAs in contrast to the ZM group. However, both SO and ZM were characterized by higher SFAs and lower UFAs. These results suggest that the fatty acid composition in the breast tissue was directly improved by the inclusion of TM oil to the broiler diet.

The PUFA and SFA ratio, which is the crucial marker of lipid composition in a healthy diet, of the SO and TM groups were characterized by results recommended for consumers: 0.93 and 0.95, respectively (Paul et al., 2017). These results suggest the possibility of reducing the risk of tumor formation or atherosclerosis (Turley and Thompson, 2015). This is in agreement with both thrombogenic and atherogenic indices which were reduced by TM oil inclusion. However, negative effects of TM oil were also observed in the decreasing n-3 and n-3 PUFA contents and, consequently, the increasing value of the n-6 to n-3 ratio. The deficiencies in n-3 fatty acids are connected with the wheat bran and wheat flour additions to the insect diets to reduce the humidity of food-waste (Bendová et al., 1991; Burkwall and Glass, 1965). Thus, it may be concluded that for future market implementation, the most suitable oil to meet consumer requirements will be TM oil. However, it must be highlighted that the insect fatty acid composition could be modified by nutrition, i.e., the rearing substrate (Makkar et al., 2014), to increase the PUFA (mainly n-3 PUFA) content. Because of this, more data are needed on improving the fat composition during insect rearing.

As was mentioned above, the liver is a metabolic center of the organism and takes a major role in fatty acid metabolism, deeply influencing fatty acid distribution in the body, i.e., meat tissues. In addition to that, the liver is also an important part of the immune system. Immune cells present in the liver are mostly in an activated state. Their major role is maintaining the immune tolerance of the organism. This activity is supported by hematopoietic cells and

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transforming growth factor-*β* (Jakab, 2015). Therefore, one of the genes which was analyzed in liver tissue was *GIMAP5*. *GIMAP* stands for "GTPase" and is in the immunity-associated protein family. Authors of a study on mutant mice with *GIMAP5*-deficient liver cells showed that those mice became lymphopenic, demonstrating a hematopoietic cell-intrinsic function for *GIMAP5*. Therefore, it has been concluded that *GIMAP5* is a key regulator of hematopoietic integrity and lymphocyte homeostasis (Barnes et al., 2010). In this study, the *GIMAP5* gene was downregulated in both experimental groups. In our opinion, these results should be considered as a beneficial effect of the TM and ZM supplementations, while downregulation of immune-related genes results in no boost of the immune system.

In an experiment on genetically lean and fat chickens, *APOA1* was identified as one of the genes responsible for phenotypic fatness variability. Fat-line chickens displayed significantly higher hepatic transcription rates and mRNA levels for *APOA1* than the lean-line chickens (Daval et al., 2000). In this study, a similar pattern of *APOA1* gene expression was observed. The experimental group supplemented with ZM had higher gene expression of *APOA1* on day 28, and at the same time, individuals from this group had higher BWG.

Hepatic *HNF4α* is essential for controlling the basal expression of numerous genes involved in lipid metabolism and is indispensable for maintaining normal lipid homeostasis (Yin et al., 2011). In an experiment performed on mice a reduced hepatic *HNF4α* expression resulted in a phenotype including: the development of a fatty liver and a >80% decrease in plasma triglycerides, total cholesterol, and high-density lipoprotein cholesterol (Yin et al., 2011). In the current study experimental group which received TM showed decreased expression of *HNF4α* gene, what might suggest the same phenotype. Decrease in plasma triglycerides and high density lipoprotein cholesterol is beneficial for the host organism and also from the consumer perspective. This is in agreement with thrombogenic and atherogenic indices

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reduced by TM supplementation. Gene expression analysis confirms different impact of each of used insects' oils on the host organism.

Conclusions

The results of the current study suggest that inclusion of TM, as well as ZM, obtained using super-critical CO_2 extraction can be used to completely replace soybean oil in broiler chicken diets without any adverse impact on the growth performance and digestibility of nutrients. Furthermore, only TM added to the basal diet positively affected the breast meat fatty acid content, which is a component of consumer quality requirements. However, some undesirable changes in the fatty acid profile suggest that further studies be performed to establish the most effective type of insect oils for chicken nutrition. It should be emphasized that results of the present study as a first report, support the statement that both oils might be considered biologically active substances which added to the chicken diet modify molecular patterns at the mRNA level.

Conflict of interest

The authors declare that there are no conflicts of interests.

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Figure caption

Figure 1. Changes in relative expression of genes in the liver of broiler chickens that received *Tenebrio molitor* oil or *Zophobas morio* oil. (Experiment 2).

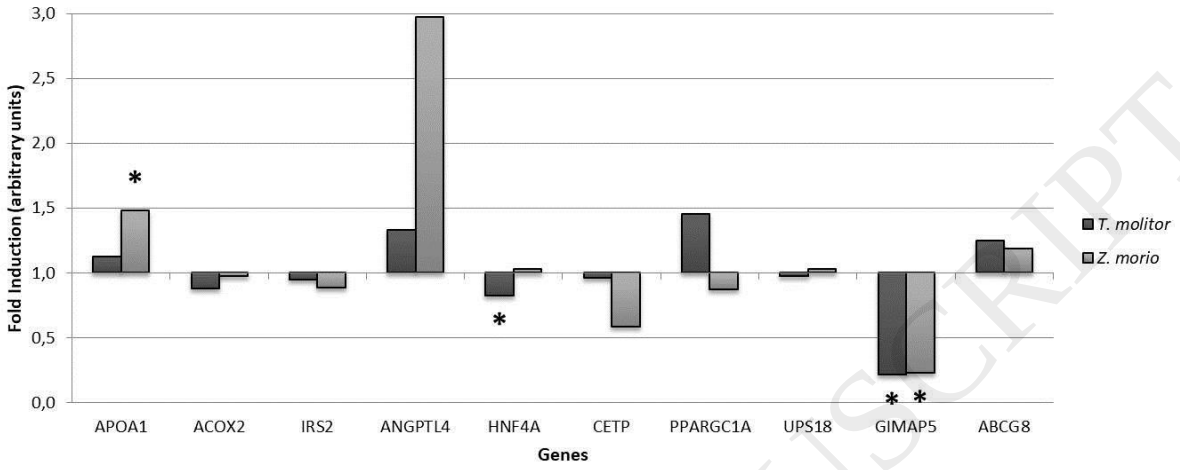

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Table 1. Composition of the basal diet (1 - 28 days).

Ingredient (g/kg)	
Maize	576.5
Soybean meal	379.6
Dicalcium phosphate	25.7
Mineral-vitamin premix[a]	3.0
Limestone	5.9
Salt (NaCl)	2.4
Sodium carbonate (Na_2CO_3)	1.3
L-Lysine	1.9
DL-Methionine	2.9
Tryptophane	0.3
Titanium dioxide (TiO_2)	3.0
Calculated nutritive value (g/kg)	
ME (MJ/kg)	11.6
Crude protein	228.0
Crude fat	27.0
Crude fiber	27.5
Sodium – Na	1.5
Calcium – Ca	9.8
P available	4.9
Lysine	13.7
Methionine	5.9
Methionine + cysteine	9.6
Threonine	8.6
Analyzed chemical composition (g/kg)	
Gross energy (MJ/kg)	17.83
Crude protein	216.2
Ether extract	75.2

[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, 1.2 mg; choline, 379 mg; D-pantothenic acid, 12.5 mg; riboflavin, 7.0 mg; niacin, 41.7 mg; thiamine, 2.2 mg; D-bioyin, 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), 0.6 mg; and Se (Na_2SeO_3), 0.3 mg.

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Table 2. Fatty acid profiles of soybean, *Tenebrio molitor* and *Zophobas morio* dietary oils (g/100 g FA).

Item	SO[1]	TM[2]	ZM[3]
Saturated			
C8:0 Caprylic	0.13	0.19	0.79
C10:0 Capric	0.13	0.14	0.28
C14:0 Myristic	0.42	2.13	1.20
C16:0 Palmitic	10.9	16.4	32.4
C18:0 Stearic	4.52	2.51	7.28
C20:0 Arachidic	0.11	0.25	0.21
Monounsaturated			
C16:1 Palmitoleic	0.03	0.76	1.36
C18:1 *c*9 Oleic	23.7	43.8	37.5
C18:1 *c*11 Elaidic	1.70	0.98	0.87
C20:1 Eicosenoic	0.11	0.14	0.09
C20:1t	0.35	0.30	0.50
Polyunsaturated			
C18:2 *c*9*c*12 Linoleic, LA	50.3	30.2	16.5
C18:3 *c*9*c*12*c*15 α-Linolenic, LNA	7.21	1.59	0.67
Others[4]	0.74	0.94	0.85
SFA[5]	16.2	21.6	42.2
UFA[6]	83.8	78.4	57.8
MUFA[7]	26.3	46.6	40.6
PUFA[8]	57.5	31.8	17.2
MCFA[9]	11.47	19.38	35.02
n-6	50.3	30.2	16.5
n-3	7.21	1.59	0.67
n6/n3	7.05	19.2	24.8
PUFA/SFA	3.55	1.47	0.41
Linolenic/Linoleic	0.14	0.05	0.04

[1]SO – soybean oil; [2]TM – *Tenebrio molitor* oil; [3]ZM – *Zophobas morio* oil; [4]Others – C18:1 *c*12, C18:1 *c*15, C20:1 *trans*; [5]SFA – saturated fatty acids; [6]UFA – unsaturated fatty acids; [7]MUFA – monounsaturated fatty acids; [8]PUFA – polyunsaturated fatty acids; [9]MCFA – medium chain fatty acids

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Table 3. The effect of *Tenebrio molitor* oil on the growth performance of broiler chickens (Experiment 1).

Item	1-7 d			7-14 d			14-21 d			21-28 d			1-28 d		
	BWG, g	FI, g	FCR, g:g	BWG,g	FI, g	FCR,g:g	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g
SO[1]	146	170	1.16	232	384	1.66	391	593	1.52	571	974	1.70	1336	2122	1.59
TM[2]	145	163	1.13	253	379	1.50	387	560	1.45	556	954	1.72	1341	2056	1.53
SEM[3]	1.13	1.78	0.02	2.87	0.81	0.02	3.02	5.67	0.02	6.41	7.56	0.02	9.01	12.99	0.01
P-value	*0.491*	*0.067*	*0.247*	*<0.001*	*<0.001*	*<0.001*	*0.545*	*0.003*	*0.035*	*0.228*	*0.197*	*0.951*	*0.991*	*0.013*	*0.034*

[1]SO – soybean oil; [2]TM – *Tenebrio molitor* oil; SEM – standard error of the mean;

[a,b] – means within a column with no common superscripts differ significantly (P<0.05)

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Table 4. The effect of insect oils on the growth performance of broiler chickens (Experiment 2).

Item	1-7 d			7-14 d			14-21 d			21-28 d			1-28 d		
	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g	BWG, g	FI, g	FCR, g:g
SO[1]	124	122	0.98[b]	324	412	1.28	493[ab]	640	1.29[b]	600[b]	1005	1.69	1541	2265	1.47
TM[2]	114	129	1.14[a]	317	404	1.28	502[a]	658	1.31[b]	632[ab]	1010	1.60	1566	2270	1.45
ZM[3]	114	128	1.13[a]	320	411	1.29	457[b]	649	1.43[a]	664[a]	1031	1.55	1555	2291	1.48
SEM	2.85	2.40	0.03	5.07	4.38	0.02	8.03	6.54	0.02	10.08	9.28	0.03	15.33	9.28	0.01
P-value	*0.253*	*0.469*	*0.048*	*0.880*	*0.747*	*0.967*	*0.041*	*0.538*	*0.024*	*0.027*	*0.509*	*0.135*	*0.819*	*0.509*	*0.757*

[1]SO – soybean oil; [2]TM – *Tenebrio molitor* oil; [3]ZM – *Zophobas morio* oil; SEM – standard error of the mean;

[a,b] – means within a column with no common superscripts differ significantly (P<0.05)

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Table 5. The effect of *Tenebrio molitor* oil on nitrogen retention and apparent digestibility of ether extract in the total digestive tract at different ages of broiler chickens (Experiment 1).

Item	7 d		14 d		21 d		28 d	
	N[3], %	EE[4], %	N, %	EE, %	N, %	EE, %	N, %	EE, %
SO[1]	58.63	87.90	63.59	91.43	60.03	90.07	70.72	95.24
TM[2]	61.64	95.87	65.70	96.46	67.11	97.60	71.71	98.20
SEM	0.34	0.83	0.47	0.68	0.38	0.89	0.54	0.72
P-value	*0.017*	*<0.001*	*0.026*	*<0.001*	*<0.001*	*<0.001*	*0.648*	*<0.001*

[1]SO – soybean oil; [2]TM – *Tenebrio molitor* oil; [3]N – Nitrogen retention; [4]EE – Ether extract

SEM - standard error of the mean;

[a,b] - means within a column with no common superscripts differ significantly (P<0.05)

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Table 6. The effect of insect oils on the apparent ileal digestibility of crude protein, ether extract and apparent metabolizable energy corrected to zero nitrogen balance of broiler chickens (Experiment 2).

Item	28 d		
	CP[4], %	EE[5], %	AME_N[6], Kcal
SO[1]	83.26	96.64	3195
TM[2]	82.95	96.14	3183
ZM[3]	83.82	96.89	3211
SEM	0.43	0.26	15.54
P-value	*0.721*	*0.515*	*0.780*

[1]SO – soybean oil; [2]TM – *Tenebrio molitor* oil; [3]ZM – *Zophobas morio* oil; [4]CP – Crude protein; [5]EE – Ether extract; [6]AME_N – Apparent metabolizable energy corrected to zero nitrogen balance; SEM – standard error of the mean;

[a,b] – means within a column with no common superscripts differ significantly ($P<0.05$)

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Table 7. The effect of selected insect oils on fatty acid profiles of liver tissue (g/100 g FA).

Item	SO[1]	TM[2]	ZM[3]	SEM	*P-value*
Total lipids (g/100 g)	3.90[b]	4.03[ab]	5.29[a]	0.290	*0.048*
Saturated					
C8:0	0.11	0.10	0.06	0.013	*0.330*
C10:0	0.10	0.11	0.08	0.014	*0.642*
C12:0	0.17	0.15	0.11	0.015	*0.240*
C14:0	0.33	0.42	0.49	0.028	*0.120*
C15:0	0.07	0.09	0.08	0.010	*0.784*
C16:0	15.57[b]	16.46[b]	18.93[a]	0.449	*0.004*
C17:0	0.14[a]	0.06[b]	0.09[ab]	0.009	*0.017*
C18:0	21.98[a]	19.21[b]	18.12[c]	0.294	*0.001*
C20:0	0.16	0.16	0.09	0.018	*0.167*
C21:0	0.33[b]	0.49[a]	0.38[ab]	0.025	*0.033*
C22:0	13.52[a]	11.33[b]	8.43[c]	0.472	*0.001*
C23:0	0.14[a]	0.10[b]	0.07[b]	0.011	*0.040*
C24:0	0.16[a]	0.07[b]	0.08[b]	0.013	*0.027*
Monounsaturated					
C14:1	0.16	0.10	0.08	0.015	*0.136*
C16:1	0.59	0.51	0.50	0.038	*0.680*
C18:1 *c*9	13.44[c]	21.55[b]	27.02[a]	1.164	*0.001*
C18:1 *c*11	1.34[b]	3.14[a]	2.99[ab]	0.194	*0.001*
C18:1 *c*12	0.30[b]	1.00[a]	0.53[ab]	0.088	*0.003*
C20:1 *trans*	0.12	0.14	0.13	0.011	*0.844*
C22:1 n-9	0.12	0.11	0.11	0.013	*0.886*
C24:1	1.90[a]	1.28[b]	0.86[b]	0.096	*0.001*
Polyunsaturated					
C18:2 *c*9*c*12	19.51[a]	16.48[b]	14.51[b]	0.462	*0.001*
C18:2 *c*9*c*15	0.27	0.25	0.19	0.023	*0.358*
C18:3 *c*9*c*12*c*15	0.58[a]	0.32[b]	0.25[b]	0.032	*0.001*
C18:3 n-6	0.48	0.47	0.64	0.038	*0.097*
C20:2	1.44	1.67	1.68	0.063	*0.335*
C20:3 n-6	0.21[a]	0.27[b]	0.14[c]	0.024	*0.047*
C20:4 n-6	0.41[a]	0.13[b]	0.18[b]	0.023	*0.001*
C20:5 n-3	0.20[a]	0.08[b]	0.08[b]	0.019	*0.037*
C22:2	0.13	0.15	0.12	0.017	*0.790*
C22:5 n-3	1.61	1.58	1.24	0.084	*0.115*
C22:6 n-3	3.60[a]	1.19[b]	0.99[b]	0.204	*0.001*

[1]SO – soybean oil; [2]TM – *Tenebrio molitor* oil; [3]ZM – *Zophobas morio* oil;

SEM – standard error of the mean;

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[a,b] – means within a column with no common superscripts differ significantly (P<0.05)

Table 8. The summarized effect of selected insect oils on the fatty acid profile of liver tissue (g/100 g FA).

Item	SO[1]	TM[2]	ZM[3]	SEM	*P- value*
[4]SFA	53.35[a]	48.97[b]	48.06[b]	0.642	*<0.001*
[5]UFA	46.65[b]	51.03[a]	51.94[a]	0.642	*<0.001*
[6]MUFA	18.22[b]	28.58[a]	32.22[a]	1.549	*<0.001*
[7]PUFA	28.43[a]	22.45[b]	19.71[c]	0.946	*<0.001*
n-6	40.55[a]	34.82[b]	29.91[c]	1.206	*<0.001*
n-3	6.25[a]	3.30[b]	2.84[b]	0.384	*<0.001*
n-6/n-3	6.53[b]	10.60[a]	10.70[a]	0.510	*<0.001*
n-6 PUFA	20.61[a]	17.27[b]	15.11[c]	0.610	*<0.001*
n-3 PUFA	5.99[a]	3.10[b]	2.62[b]	0.376	*<0.001*
PUFA/SFA	0.53[a]	0.46[b]	0.41[c]	0.014	*<0.001*
LNA/LA	0.03	0.02	0.02	0.002	*0.050*
Total C18:1	15.08[b]	26.49[a]	30.31[a]	1.683	*0.001*
[8]MCFA	17.47[b]	18.30[b]	21.33[a]	0.564	*0.005*
[9]LCFA	82.19[a]	81.45[a]	78.53[b]	0.552	*0.007*
Δ9 (16:1/16:0)	0.033	0.028	0.033	0.003	*0.661*
Δ9 (18:1/18:0)	0.688[c]	1.382[b]	1.656[a]	0.107	*<0.001*
Δ9 (MUFA/SFA)	0.342[b]	0.588[a]	0.671[a]	0.037	*<0.001*
Δ5, n-6 (20:4n-6/20:3n-6)	2.167	1.193	2.043	0.222	*0.151*
Δ6, n-6 (20:4n-6/18:3n-6)	0.956[a]	0.225[b]	0.281[b]	0.456	*<0.001*
Δ4, n-3 (22:6n-3/22:5n-3)	2.405[a]	0.907[b]	0.778[b]	0.214	*<0.001*
Thrombogenic index	1.01[b]	1.13[a]	1.22[a]	0.027	*<0.001*
Atherogenic index	0.37	0.37	0.42	0.012	*0.072*

[1]SO – soybean oil; [2]TM – *Tenebrio molitor* oil; [3]ZM – *Zophobas morio* oil; [4]SFA – saturated fatty acids; [5]UFA – unsaturated fatty acids; [6]MUFA – monounsaturated fatty acids; [7]PUFA – polyunsaturated fatty acids; [8]MCFA – medium chain fatty acids; [9]LCFA – long chain fatty acids; SEM – standard error of the mean;

[a,b] – means within a column with no common superscripts differ significantly (P<0.05)

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Table 9. The effect of selected insect oils on fatty acid profiles of breast meat (g/100 g FA).

Item	SO[1]	TM[2]	ZM[3]	SEM	*P- value*
Total lipids (g/100 g)	1.43	3.20	3.41	0.380	*0.058*
Saturated					
C8:0	0.14	0.10	0.12	0.023	*0.759*
C10:0	0.19	0.08	0.16	0.021	*0.083*
C12:0	0.24	0.22	0.24	0.021	*0.922*
C14:0	0.79	0.94	0.91	0.049	*0.447*
C15:0	0.09	0.11	0.08	0.015	*0.646*
C16:0	18.10^{b}	16.69^{c}	21.17^{a}	0.499	*<0.001*
C17:0	0.14	0.11	0.07	0.016	*0.227*
C18:0	10.33	8.29	9.04	0.362	*0.055*
C20:0	0.20	0.11	0.07	0.026	*0.119*
C21:0	0.19	0.16	0.34	0.025	*0.145*
C22:0	6.38^{a}	5.08^{ab}	4.33^{b}	0.333	*0.036*
C23:0	0.10^{b}	0.11^{b}	0.20^{a}	0.014	*0.001*
C24:0	0.17	0.09	0.20	0.029	*0.296*
Monounsaturated					
C14:1	0.14	0.08	0.05	0.027	*0.366*
C16:1	0.54^{b}	0.68^{b}	0.88^{a}	0.044	*0.0036*
C18:1 *c*9	22.80^{b}	30.06^{a}	32.44^{a}	1.128	*<0.001*
C18:1 *c*11	2.70	3.79	2.33	0.275	*0.061*
C18:1 *c*12	0.46	0.83	0.45	0.116	*0.308*
C20:1 *trans*	0.15	0.18	0.17	0.018	*0.814*
C22:1 n-9	0.17	0.09	0.05	0.029	*0.246*
C24:1	2.23^{a}	1.73^{ab}	1.41^{b}	0.121	*0.015*
Polyunsaturated					
C18:2 *c*9*c*12	27.49^{a}	25.70^{a}	21.75^{b}	0.892	*0.021*
C18:2 *c*9*c*15	0.39	0.24	0.29	0.032	*0.174*
C18:3 *c*9*c*12*c*15	1.84^{a}	1.28^{b}	0.86^{c}	0.112	*<0.001*
C18:3 n-6	0.35^{b}	0.51^{a}	0.26^{b}	0.041	*0.024*
C20:2	0.96	0.81	0.75	0.066	*0.446*
C20:3 n-6	0.31	0.17	0.12	0.033	*0.079*
C20:4 n-6	0.35^{a}	0.16^{b}	0.23^{b}	0.028	*0.010*
C20:5 n-3	0.15	0.13	0.10	0.015	*0.440*
C22:2	0.13^{b}	0.11^{b}	0.23^{a}	0.021	*0.039*
C22:5 n-3	0.59	0.60	0.45	0.035	*0.122*
C22:6 n-3	1.15^{a}	0.62^{b}	0.46^{b}	0.083	*0.001*

[1]SO – soybean oil; [2]TM – *Tenebrio molitor* oil; [3]ZM – *Zophobas morio* oil;

SEM – standard error of the mean;

^{a,b} – means within a column with no common superscripts differ significantly (P<0.05)

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Table 10. The summarized effect of selected insect oils on fatty acid profiles of breast meat (g/100 g FA).

Item	SO[1]	TM[2]	ZM[3]	SEM	*P- value*
[4]SFA	37.02[a]	32.10[b]	36.69[a]	0.854	*0.016*
[5]UFA	62.98[b]	67.90[a]	63.29[b]	0.855	*0.016*
[6]MUFA	29.32[b]	37.56[a]	36.71[a]	1.010	*<0.001*
[7]PUFA	33.67[a]	30.34[a]	26.58[b]	0.937	*0.004*
n-6	56.52[a]	53.19[a]	44.79[b]	1.8	*0.017*
n-3	4.13[a]	2.87[b]	3.24[b]	0.159	*0.001*
n-6/n-3	13.82[b]	18.51[a]	14.41[b]	0.774	*0.012*
n-6 PUFA	28.45[a]	26.55[a]	22.36[b]	0.908	*0.014*
n-3 PUFA	3.74[a]	2.63[b]	2.96[b]	0.144	*0.001*
PUFA/SFA	0.93[a]	0.95[a]	0.73[b]	0.040	*0.037*
LNA/LA	0.07[a]	0.05[b]	0.04[b]	0.003	*0.001*
Total C18:1	25.95[b]	34.69[a]	35.22[a]	1.139	*<0.001*
[8]MCFA	20.00[b]	18.83[b]	23.38[a]	0.516	*<0.001*
[9]LCFA	79.67[a]	80.99[a]	76.33[b]	0.530	*<0.001*
Δ9 (16:1/16:0)	0.03[b]	0.03[ab]	0.04[a]	0.002	*0.025*
Δ9 (18:1/18:0)	2.86	3.62	3.22	0.134	*0.083*
Δ9 (MUFA/SFA)	0.85[b]	1.04[a]	0.88[b]	0.026	*0.006*
Δ5, n-6 (20:4n-6/20:3n-6)	0.09	0.16	0.17	0.014	*0.052*
Δ6, n-6 (20:4n-6/18:3n-6)	0.88[ab]	0.48[b]	1.29[a]	0.124	*0.023*
Δ4, n-3 (22:6n-3/22:5n-3)	1.78[a]	1.03[b]	0.98[b]	0.082	*<0.001*
Thrombogenic index	0.73[ab]	0.65[b]	0.81[a]	0.024	*0.011*
Atherogenic index	0.34[b]	0.30[b]	0.40[a]	0.012	*0.001*

[1]SO – soybean oil; [2]TM – *Tenebrio molitor* oil; [3]ZM – *Zophobas morio* oil; [4]SFA – saturated fatty acids; [5]UFA – unsaturated fatty acids; [6]MUFA – monounsaturated fatty acids; [7]PUFA – polyunsaturated fatty acids; [8]MCFA – medium chain fatty acids; [9]LCFA – long chain fatty acids;

SEM – standard error of the mean;

[a,b] – means within a column with no common superscripts differ significantly (P<0.05)

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