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SHORT COMMUNICATION

Fatty acid profile of lipids and caeca volatile fatty acid production of broilers fed a full fat meal from *Tenebrio molitor* larvae

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ABSTRACT

Volatile fatty acid production (VFA) in the caeca and fatty acid profile of meat and fat depots were investigated on 62 days old broiler equally divided in 2 groups fed with a corn-soybean (SBM) or a corn-insect meal (TML, from *Tenebrio molitor* larvae) based diet from 30 days of age. The total amount of VFAs was higher ($p < .01$) in broilers fed TML and the same happened for all the single VFA studied in the trial. When the VFAs were expressed as percentage of total VFAs, acetate, propionate and valerianate were higher ($p < .01$) in SBM group, while the proportion of butyrate was higher ($p < .01$) in broilers fed TML. The fatty acids C12:0 and C14:0 had higher percentages ($p < .01$ and $p < .05$, respectively) in the intramuscular fat of broilers fed TML diet. Regarding the FA groups (SFA, MUFA and PUFAs), only the PUFAn1 fatty acids were higher ($p < .01$) in the broilers fed TML diet. Insect meals can affect the microbial activity in broiler caeca and, in addition, induced very few modifications in the fatty acid profile of broiler breast.

HIGHLIGHTS

- *Tenebrio molitor* in broiler feeding affects the production of volatile fatty acids (VFA) in the caeca.
- The total production of VFAs doubled in *Tenebrio molitor* feeding group, and the butyrate increased (+183%).
- TM slightly modify the fatty acid profile of breast meat.

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Broiler; Tenebrio molitor; volatile fatty acids; fatty acid profile of lipids

Introduction

Along the last years, insect meals are strongly investigated as potential protein source in broiler feed (Bovera et al. 2016; Józefiak et al. 2016; Schiavone et al. 2017a), showing positive effects on growth performance and health status of the birds, but having a negative effect on nutrient digestibility of the diets, probably due to the role of the chitin contained in the insect exoskeleton (Bovera et al. 2015). Chitin could be an interesting compound because it seems to have an important role in improving the health status of poultry fed with diet containing insect (Cullere et al. 2016). Borrelli et al. (2017) showed that hens feeding *Hermetia illucens* larvae meal as full replacement of soybean meal had a higher production of butyric acid in the caeca, due to the modification of the microbiota, and this volatile fatty acid is known to have a protective role on intestinal mucosa (Loponte et al. 2017).

More recently, the interest in evaluating the effect of insect fats from *Hermetia illucens* (Schiavone et al. 2018) or other insect larvae, such as those of *Tenebrio molitor* and *Zophobas morio* (Kierończyk et al. 2018) is increasing. However, the insect fat can markedly affect the fatty acid profile of the meat and of the fat depots in poultry, considering that the diet composition is the main factor affecting the fatty acid profile of the poultry lipids (Crespo and Esteve-Garcia 2001), with important consequences under nutritional point of view for human (lipid of meat) and pets (lipid depots),

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considering that lipid depots of poultry are largely used in pet food formulation.

This study represents the continuation and completion of a previous trial (Bovera et al. 2016) in which the growth performance and meat quality of broilers fed *T. molitor* larvae meal from 30 to 62 days of age has been investigated. Thus, the aim of the present research was to evaluate the effect of the inclusion of a full fat meal from *Tenebrio molitor* larvae in the diet on the volatile fatty acid production in the caeca and on the fatty acid profile of the meat and fat abdominal depots of 62 days old broilers.

Material and Methods

All the animals were treated according to the principles stated by the Directive 2010/63/EU regarding the protection of animals used for experimental and other scientific purposes. The study was carried out on a private poultry farm of the province of Caserta (Italy) and the experimental procedures received the approval from the Ethical Committee of the Department of Veterinary Medicine and Animal Production of the University of Napoli Federico II (Italy).

A total of 80, 30 days old male Shaver brown broilers (average body weight 1.76 ± 0.19 kg) were homogenously divided into 2 groups (40 birds per group, each consisting of 8 replicates of 5 birds each), housed in a semi-opened building. Each replicate was placed in a floor pen (1.0 x 1.0 m/pen) furnished with rice hulls as litter. Up to 62 days of age, the groups were fed two isoproteic and isoenergetic diets, whose ingredients and chemical-nutritional characteristics are reported in Table 1 while their fatty acid composition is summarized in Table 2. The control group was fed a corn-soybean meal (SBM) based diet, while for the other group the soybean meal was completely replaced by *Tenebrio molitor* larvae meal (TML, Gaobeidian Shannon Biology CO., Ltd., Shannong, China). The diets were formulated to meet poultry requirements according to NRC (1994) and provided *ad libitum*. At 62 days of age, two broilers per replicate (16 per group) were weighed and slaughtered in a specialized slaughterhouse. From each carcass, the right breast and the abdominal fat were collected and stored at −80 °C until analysis. The caeca were tied at both ends, separated by sterile instruments, placed in tightly closed plastic bags and put in pre-warmed thermos. After sampling, the material was transported as soon as possible (about 1 h) to the laboratories where two quotes of caecal content (each about 5 ml) were utilized for volatile fatty acids (VFAs) analysis. After dilution of the samples with oxalic acid (1:1, v/v), the VFAs were analyzed by a gas chromatograph method as described by Bovera et al. (2010).

Table 2. Fatty acid profile (% of total fatty acids) of *Tenebrio molitor* larvae meal (TM) and experimental diets (SBM and TML).

	TM	SBM	TML
C14:0	2.15	0.12	2.42
C16:0	16.69	14.99	18.25
C18:0	3.46	3.25	3.84
C16:1n7	1.56	0.15	1.68
C18:1n9	31.56	33.25	38.12
C18:1n7	0.56	1.85	1.39
C18:2n6	36.42	44.56	30.35
C18:3n3	2.34	2.41	2.39
C22:6n3	1.07	2.54	1.95
SFA	23.19	18.36	24.51
MUFA	35.11	36.81	41.19
PUFAn6	36.71	44.56	30.35
PUFAn3	4.68	4.95	4.34

The fatty acids C20:1n9, C22:1n11, C18:4n3, C20:5n3, C22:5n3, found in the samples in percentage lower than 1%, were not included in table but considered in the fatty acid fractions.
SBM: soybean meal group; TML: Tenebrio molitor larvae meal group.

Table 1. Chemical-nutritional characteristics of the diets used in the trial.

	Soybean meal diet	*Tenebrio molitor* larvae meal diet
Dry matter, %	86.62	88.14
Crude protein, % AF	20.36	20.19
Ether extract, % AF	4.75	5.23
Crude fiber, % AF	4.11	3.93
Ash, % AF	6.77	6.67
Lysine, % AF	1.24	1.18
Methionine + Cystine, % AF	0.89	0.96
Trypsin, % AF	0.24	0.27
Threonine, % AF	0.81	0.90
Arginine, % AF	1.49	1.32
ME, kcal/kg	2,812	2,849

AF: as feed; ME: metabolizable energy.
Ingredients
soybean meal diet (g): corn grain (438.1), soybean meal 44% (446.5), vegetable oil (22.0), Monocalcic phosphate Cerqual (9.50), MinVit (6.40), NaCl (4.24), Calcium Bicarbonate (7.44), DL-Methionine (2.67), Sodium Bicarbonate (2.50), Fe (0.70), L-lysine (0.30).
Tenebrio molitor larvae meal (g): corn grain (641.4), Insect meal (296.5), Monocalcic phosphate Cerqual (9.50), MinVit (6.40), NaCl (4.24), Calcium Bicarbonate (7.44), DL-Methionine (3.67), Sodium Bicarbonate (2.50), Fe (0.70), L-lysine (0.30.)

Total lipid extraction from breast and abdominal fat was performed according to a modified Folch et al. (1957) method. The extracted lipids were utilized for the analysis of fatty acid (FA) profile. In particular, the FAME (Fatty Acid Methyl Ester) analysis was performed by gas-chromatography according to a modified method of Morrison and Smith (1964), as detailed in Secci et al. (2016). Fatty acids were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix (Supelco, Bellefonte, PA, USA). Fatty acids were quantified through calibration curves, using tricosanoic acid (C23:0) (Supelco) as internal standard. For the evaluation of the FA profile in terms of human nutrition and health, the FA composition was utilized to calculate the atherogenicity index (AI) and thrombogenicity index (TI), according to Ulbricht and Southgate (1991), and the hypocholesterolemic to hypercholesterolemic fatty acids ratio (h/H), according to Santos-Silva et al. (2002), using the following formulas:

$$AI = \frac{[C12:0 + (4 \times C14:0) + C16:0]}{MUFA + PUFAn6 + PUFAn3}$$

$$TI = \frac{(C14:0 + C16:0 + C18:0)}{[(0.5 \times MUFA) + (0.5 \times PUFAn6) + (3 \times PUFAn3) + (PUFAn3/PUFAn6)]}$$

$$h/H = \frac{(C18:1cn9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:6n3)}{(C14:0 + C16:0)}$$

Moreover, the ratios PUFAn6/PUFAn3 and PUFA/SFA were calculated. For the quality index calculation, the fatty acids concentrations were expressed as g/100 g of sample.

Data were analyzed by one-way ANOVA using the GLM procedure of SAS (2002), according to the model:

$$Yij = m + Pi + eij$$

where Y: the single observation, m: the general mean, P: the effect of protein source (i = soybean meal or *Tenebrio molitor* larvae meal), and e: the error. Differences among means were separated using Tukey's test (SAS 2002) at $p < .05$.

Results

Table 3 shows the effect of TML diet on volatile fatty acid production in caecum, expressed both as mmol/l or percentage of the total measured VFAs. The total amount of VFAs was higher ($p < .01$) in broilers fed TML in comparison to SBM group and the same happened for all the single VFA studied in the trial. When the VFAs were expressed as percentage of total VFAs, acetate, propionate and valerianate were higher ($p < .01$) in SBM group, while the proportion of butyrate was higher ($p < .01$) in broilers fed TML. No differences were found between the relative proportion of isobutyrate and isovalerianate.

Table 4 reports the fatty acid profile of the breast

Table 3. Volatile fatty acids (VFAs) level in the caecal content of broilers fed soybean or insect meal-based diet at 62 days of age.

	SBM	TML	RMSE	*p*-value
Mmol/l				
Acetate	6.64^{B}	11.13^{A}	2.83	.0002
Propionate	2.64^{B}	4.49^{A}	1.27	.0005
Isobutyrate	0.26^{B}	0.48^{A}	0.19	.0036
Butyrate	1.44^{B}	4.07^{A}	0.94	<.0001
Isovalerianate	0.38^{B}	0.79^{A}	0.38	.0072
Valerianate	0.27^{b}	0.41^{a}	0.15	.0189
Total VFAs	11.63^{B}	21.38^{A}	5.22	<.0001
% of total VFAs				
Acetate	56.86^{A}	52.30^{B}	3.64	.0023
Propionate	22.78^{A}	2.91^{B}	1.60	.0040
Isobutyrate	2.31	2.20	0.65	.6642
Butyrate	12.23^{B}	19.19^{A}	3.02	<.0001
Isovalerianate	3.49	3.55	1.23	.9069
Valerianate	2.33^{A}	1.85^{B}	0.38	.0022

SBM: soybean meal group; TML: *Tenebrio molitor* larvae meal group; RMSE: root mean square error; $^{a,b}p < .05$; $^{A,B}p < .01$.

Table 4. Fatty acids profile (as % of total fatty acids) of the broiler breast.

	SBM	TML	RMSE	*p* value
Fatty acids				–
C12:0	1.26^{b}	1.37^{a}	0.11	.002
C14:0	1.86^{b}	1.98^{a}	0.18	.040
C16:0	18.52	18.06	0.88	.106
C16:1n7	3.07	2.95	0.66	.58
C18:0	6.54	6.52	0.49	.856
C18:1n9	22.93	22.33	2.47	.437
C18:1n7	2.79	2.88	0.19	.105
C18:2n6	22.15	22.00	1.88	.806
C18:3n3	1.76	1.79	0.21	.664
C20:3n6	0.98	1.00	0.19	.705
C20:4n6	4.49	4.48	1.12	.987
C22:4n6	1.33	1.36	0.39	.793
SFA	31.57	31.62	1.08	.891
MUFA	32.31	31.98	2.79	.711
PUFAn6	30.61	30.62	2.34	.983
PUFAn3	4.93	5.08	0.56	.391
PUFAn1	0.59^{b}	0.70^{a}	0.10	.001
Quality indexes				
PUFAn6/PUFAn3	6.26	6.07	0.56	.2771
Atherogenicity index	1.06	1.09	0.07	.159
Thrombogenecity index	0.35	0.35	0.06	.983
h/H	2.58	2.59	0.19	.827

The fatty acids C13:0, C14:1n5, C15:0, C16:1n9, C17:0 anteiso, C17:0, C17:1, C16:4n1, C18:3n6, C18:4n3, C20:0, C20:1n11, C20:1n9, C20:1n7, C20:2n6, C20:3n3, C20:4n3, C20:5n3, C22:0, C22:5n6, C22:5n3, C22:6n3, found in the samples in percentage lower than 1%, were not included in the table but considered in the fatty acid fractions.

h/H: hypocholesterolemic to hypercholesterolemic fatty acids ratio; SBM: soybean meal group; TML: Tenebrio molitor larvae meal group; RMSE: root mean square error; $^{a,b}p < .05$.

Table 5. Fatty acids content (as % of total fatty acids) of the broiler abdominal depot.

	SBM	TML	RMSE	*p*-value
Fatty acids				
C12:0	1.27	1.31	0.14	.545
C14:0	1.80	2.01	0.19	.056
C16:0	17.01	16.39	2.58	.662
C16:1n7	4.34	3.91	0.81	.343
C18:0	4.81	4.80	0.77	.990
C18:1n9	27.49	27.85	1.80	.709
C18:1n7	2.70	2.61	0.20	.421
C18:2n6	25.76	26.30	2.38	.680
C18:3n3	2.53	2.28	0.26	.083
SFA	28.42	28.17	2.98	.878
MUFA	38.15	38.10	2.68	.973
PUFAn6	28.51	29.05	2.49	.693
PUFAn3	4.32	4.06	0.35	.191
PUFAn1	0.61	0.63	0.06	.510

The fatty acids C13:0, C14:1n5, C15:0, C16:1n9, C17:0 anteiso, C17:0, C17:1, C16:4n1, C18:3n6, C18:4n3, C20:0, C20:1n11, C20:1n9, C20:1n7, C20:2n6, C20:3n6, C20:4n6, C20:3n3, C20:4n3, C20:5n3, C22:0, C22:4n6, C22:5n6, C22:5n3, C22:6n3, found in the samples in percentage lower than 1%, were not included in the table but considered in the fatty acid fractions.
SBM: soybean meal group; TML: Tenebrio molitor larvae meal group; RMSE: root mean square error.

obtained from the broilers of the two experimental groups slaughtered at 62 days of age. The fatty acids C12:0 and C14:0 had higher percentages ($p < .01$ and $p < .05$, respectively) in the intramuscular fat of broilers fed TML diet. Regarding the FA groups (SFA, MUFA and PUFAs), only the PUFAn1 fatty acids were higher ($p < .01$) in the broilers fed TML diet.

None of the criteria presented in Table 5 (fatty acid profile of broiler abdominal fat) was affected by dietary treatment.

Discussion

As reported in our previous study (Bovera et al. 2016), broilers fed *T. molitor* had no different final weight (3.47 vs. 3.37 kg), body weight gain (53.40 vs. 50.49 g/d), feed intake (192.4 vs. 207.8 g/d), and chemical traits of breast meat in comparison to the control group.

The effect of TML diet on VFAs profile of broiler caeca was evident and indicated an almost doubled total production of VFAs in this group than the control and, specifically, the butyrate showed a very important increase (+183%) in comparison to the SBM group. Butyrate is considered the prime enterocytes energy source (Bovera et al. 2010) and it is also necessary for the suitable development of the Gut-Associated Lymphoid Tissue (Mroz 2005). It is documented that butyrate is the major intestinal energy source even when other fuel sources (glucose or glutamine) are available and could stimulate the growth of colorectal and ileal mucosal cells (Topping and Clifton 2001; Montagne et al. 2003). This effect is important for maintaining the function of the full gastro-intestinal tract (Montagne et al. 2003). When a higher amount of butyrate is available, the increase of nutrients for enterocytes enhances blood flow through intestine and then the tissue oxygenation and the nutrient transport and absorption (Mahdavi and Toki 2009). Van der Wielen et al. (2000) reported that volatile fatty acids have a bacteriostatic effect on some enteric bacteria, including *Salmonella typhimurium*, and they do not inhibit the beneficial gastrointestinal tract bacteria, such as *Lactobacillus*, in chickens. A significant negative correlation between caecal propionate concentrations and *Salmonella* colonization in young chickens has been also reported (Nisbet et al. 1996). Moreover, van der Wielen et al. (2000) reported that the increased concentrations of butyrate were correlated to decreased amounts of *Enterobacteriaceae*. Therefore, the high concentration of butyrate produced in the caeca of broilers fed TML diet may play an important part in the mechanism that inhibits *E. coli* and *Salmonella* colonization.

Unfortunately, sufficient information in literature on the effects of feeding insect meal on intestinal microflora and volatile fatty acids production of broiler is not available. However, our results could be likely ascribed to the amount of chitin assumed by broilers of TML group, due to its ability to act as prebiotic in broilers (Bovera et al. 2015). To confirm this, Khempaka et al. (2011) reported that the inclusion of shrimp head meal (another chitin source) at 15 or 20%, as well as the addition of 1.9% of purified chitin in broiler diet significantly increased the production of butyric volatile fatty acid in caeca and, even if not significant, there was also an increase in acetate and propionate when the inclusion level of purified chitin increased from 1.9 to 3.8%. Standing to our estimation, the amount of chitin in the insect meal used in the present trial was 4.62% as feed, corresponding to 64.2% of ash free acid detergent fiber (ADF), in line with the finding of Finke (2007) who indicated that ADF fraction in insects contains an amount of protein from 9.3 to 32.7% and the amount of chitin ranges from 2.7 to 49.8 g/kg. In a recent research, Borrelli et al. (2017) stated that the microbiota plays a key role in the coordination of polysaccharide degradation responsible for the increase in short chain fatty acids (SCFAs) concentrations in the gut of laying hens fed *Hermetia illucens* larvae meal.

In the present trial, even if the broilers of the two groups were fed diets with a similar amount of lipids, in the SBM group the main source of fat was the vegetable oil, while in the TML group was the full fat

Tenebrio molitor larvae meal that contained around 22% of ether extract (Bovera et al. 2016) and thus the diet did not contain vegetable oil. The higher level of C14:0 detected in the breast of broilers fed TML diet reflected the amount of this specific fatty acid in the diet, higher than in the control one. However, despite the great differences between the diets, in particular for SFA and PUFAn6 (+33.50 and −31.89% in the TML diet, respectively), no differences were observed for the main groups of fatty acids (SFA, MUFA and PUFA) between the breast meat from the two dietary treatments and the same was also found for the lipid quality indexes. In addition, also the fat depot obtained from abdomen was unchanged for fatty acid profile in broilers fed soybean or insect meal diet. Since dietary lipids markedly influence the fatty acid composition of meat and adipose tissue in broilers (Crespo and Esteve-Garcia 2001), these results are not easy to explain and are in partial disagreement with previous findings in literature. Schiavone et al. (2017b) observed that the supplementation of *H. illucens* oil to broiler diet increased SFA and decreased PUFA in the breast muscle but did not affect MUFA content, while Kierończyk et al. (2018) observed a decrease of SFA and an increase of MUFA in the breast of broilers fed TM oil, but the level of PUFA was unchanged in comparison to the broilers fed soybean oil. The disagreement between the results of other trials using the same insect species could be ascribed to the well documented variability of the fatty acid composition of the insect fat, due to the rearing substrate utilized for larvae production (Makkar et al. 2014).

Conclusions

The insect meal from *Tenebrio molitor* larvae can be consider in poultry nutrition not only for its protein content but also for the effects on caecal microbial activity, as it is can have the potential to improve the health condition of the gastro-intestinal tract. In addition, even if from animal origin, the fat contained in the *Tenebrio molitor* larvae meal used in this trial did not modify the fatty acid profile of lipid depots and, more interesting, very few changes were detected in the fatty acid profile of breast meat. This last aspect is very important as the nutritional properties of the meat were unchanged. However, further studies need to evaluate the effect of the raising substrate of the insect on these effects.

Disclosure statement

No potential conflict of interest was reported by the authors.

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