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Insect-based diets effects on turkey meat quality

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Abstract

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Data on the effects of insect-based diets on turkey meat quality is limited. To address this, the current study aimed to assess the effects of 10% inclusion of insect meals from Black soldier fly (*Hermetia illucens*) and Silkworm (*Bombyx mori*) on turkey meat quality by analyzing physicochemical (pH, water holding capacity, cooking loss, color) and nutritional parameters (protein, amino acids, lipid and mineral content). Hybrid female turkeys at 56 day of age were divided into five dietary treatments: Control (soybean meal), Silkworm meal (SW), Silkworm meal with probiotic mix 'Zoovit' (SWpro), Black soldier fly defatted (BSFd) and Black soldier fly whole larvae (BSFw) meals. The experiment lasted for 74 days, from 56-130 days of a turkey's age. Overall results suggest that 10% inclusion of insect meal have positive effects on turkey meat, especially on technical parameters, such as water holding capacity and cooking loss. Breast and thigh meat responded differently to the diets. Breast meat had significant physicochemical responses to the new diets (improved water holding capacity, cooking loss), but lacked nutritional – no change in protein, lipid, or mineral content. In contrast, in the thigh, both BSF diets increase the level of lipids and Iron. Insect diets also improve omega-3 and omega-6 fatty acid levels. The applicability of the results is demonstrated by the fact that insect meals can alter turkey meat quality in a tissue-specific manner.

Keywords: Black soldier fly (*Hermetia illucens*); Silkworm (*Bombyx mori*); insect-based diets; turkeys; feeding; meat quality

Introduction

Turkey meat is an excellent source of nutrients with a high protein-low fat content, as well as a wide range of health promoting components like microelements (eg. selenium, with immunomodulatory functions), vitamins, and essential amino acids, including high levels of tryptophan. Tryptophan is a precursor for synthesis of serotonin, which plays diverse biological roles as neurotransmitters, neuromodulators and hormones (Petrova & Moffett, 2016). The nutritional qualities of turkey meat have been drawing attention to consumers, whose increasing awareness of choosing healthy and high-quality food have impacted the rising global production of turkeys. Poultry diets have a direct impact on birds' welfare and quality of meat. In recent years, the idea of including insect-based diets into poultry production is drawing attention, and several research investigations indicated the benefit of this. Insect meals are rich in proteins and fats with a balanced spectrum of essential amino acids, an important factor in poultry nutrition. In addition, insects are rich in a wide range of health-promoting components such as chitin, lauric acid, and antimicrobial peptides (Gasco et al., 2018) that can impact birds' growth and welfare. To date, several insect species such as Black soldier fly (Pasotto et al., 2020), Silkworm (Ullah et al., 2017), grasshoppers (Sun et al., 2012), house flies (Radulović et al., 2018), and mealworms (Elahi et al., 2020) have been investigated as a potential feed for poultry and livestock.

Data on the effects of insect-based diets on turkey growth and production is limited. Our previous investigations on inclusion of 10% insect meals from Black soldier fly (*Hermetia illucens*) and Silkworm (*Bombix mori*) into turkeys' diet have demonstrated diet-dependent differential effects on turkeys' production parameters – live weight (LW), average daily gain (ADG), daily feed intake (DFI) and feed conversion ratio (FCR), but no effects on the overall physiological status (Lalev et al., 2020). To further understand the impact of insect-based diets, the study focused on analyzing turkey meat quality by establishing the physicochemical (pH, water holding capacity, cooking loss, color) and nutritional parameters (protein, amino acids, lipid and mineral content).

Material and Methods

Birds and husbandry

The present study was conducted at the Poultry farm of the Agricultural Institute, Stara Zagora, Bulgaria. All procedures including the use of birds, management and care were in compliance with the European Council Directive regulations on the protection of animals used for experimental and other scientific purposes (2010/63/EU), and National Protocol (№ 20 from 01.11.2012).

The experimental design of the present study is reported in Lalev et al. (2020). In brief, a total of 75 female Hybrid turkeys at 56 day of age were assigned to five dietary treatments (15 birds/group) and raised from 56 to 130 days of age. Five diets were set up, where in the control group turkeys were fed with a standard soybean-based diet (SBM), and in the treatment groups insect meals were included as follow: SW – 10% silkworm meal, SWpro – 10% silkworm meal supplemented with probiotic Zoovit (0.05%, containing *Lb. Bulgaricus, Lb. Acidophilus, Str. Termophilus, L. Lactis, Propionibacterium*), BSFd – 10% defatted BSF larvae meal, BSFw – 10% BSF whole larvae meal. The diets were formulated to meet nutritional requirements of birds according to the nutrition recommendation for Hybrid commercial turkeys (2019). All diets were isonitrogenous and isocaloric. The feeding program covered four feeding periods adjusted to the age and developmental stage of the birds.

Insect meals

The BSF meals (whole larvae and defatted) used in the study were produced and provided by 'NASEKOMO', Bulgaria. The spent silkworm pupae (chrysalis, *Bombix mori* L.) were obtained from the Scientific Center of Sericulture, Vratsa, Bulgaria. Chemical analyses of all insect meals (SW, BSFd, BSFw) were carried out at the University of Food Technologies in Plovdiv, Bulgaria. Moisture and fat contents were determined according to the Association of Official Analytical Chemists methods 925.09 and 922.06, respectively (Association of Official Analytical Chemists [AOAC] 2006). Crude protein was determined by the Kjeldahl method (984.13). Amino acid analyses were performed using HPLC Waters AccQ Tag Method.

Physicochemical analysis of meat

At 130 d of age, six birds from each dietary treatment group were chosen on the basis of the average body weight and were slaughtered after 12 h of fasting. The breast and leg meat samples were used to determine pH value, meat colour, water capacity and cooking loss. Muscle pH was determined by an electronic pH meter (Testo 205) at 24 h post-mortem. The color was measured on the fresh breast and leg meat of each carcass using Minolta CR-410 colorimeter (Konica Minolta, Japan) in D-65 lighting, with a standard angle of 2° of shelter and 8 mm aperture of the measuring head. Results were described in the CIE-Lab system as lightness (L*), redness (a*), and yellowness (b*). Water holding capacity (WHC) was determined based on the classical method of Grau & Hamm (1953). Cooking loss of breast and leg meat was determined in an air convection oven. For this purpose, samples with approximate size 2/2 cm were weighed with precision of 0.01 g and cooked in an oven previously heated to 150°C for 20 min. The method principle was based on attaining a temperature of 75–80°C in the core of the sample (Petracci & Baeza, 2011). The cooking loss was calculated based on the weight loss that took place during cooking as a percentage of the initial weight.

Meat quality

Breast and leg meat samples were collected to determine their amino acid profile, fatty acid composition, mineral composition (Fe, Ca, Mg, P), and cholesterol level. The laboratory analyses were carried out in the officially accredited food laboratory Alimenti (D & V Consult Ltd.), Plovdiv, Bulgaria.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using STATISTICA software ver. 10 (Statsoft, Inc., 2011), according to the following model: $Yij = \mu + CPi + eij$, in which Y is the single observation, μ is the general mean, CP is the effect of diet (i = SBM; SWM; BSFd; BSFw) and *e* is the error. Normality and homoscedasticity tests were run on data before the means were tested and the significance was set to P<0.05. For parametric analysis, Fisher's least significant difference (LSD) test was used to compare mean group differences, while for nonparametric analysis, was used the Kruskal-Wallis test. The results were expressed as mean and pooled standard error of the mean and P<0.05 was considered statistically significant.

Results

Insect meal analyses are present in Table 1 and used for the preparation of turkeys' isocaloric diets (Table 2). Health and mortality events are monitored daily, and during the experimental period no mortalities occurred. The new diets had some limited effects on the pH_{24} values, and these were recorded only in turkeys fed with SW and SWpro diets. Small, but significant responses were recorded in the breast of SWpro group (Table 3; 5.74 vs. control 5.67; *P* = 0.006), and in the thigh of the SW fed turkeys (5.70 vs. the control 5.92; *P* = 0.001). The analysis of the next parameter, the water holding capacity (WHC), suggested that the tested diets had rather positive effects.

Table 1. Chemical composition of insect meals

Parameters	SW	BSFd	BSFw
Fat content, %	24.50	7.79	13.48
Protein content, %	57.14	56.16	44.76
Moisture content, %	11.50	1.03	0.83
Gross energy, kcal/kg	5831	2090	2325
Calcium, %	0.55	0.84	0.18
Phosphorus, %	0.75	0.67	0.43
Amino acid content, %			
Valine	5.60	4.79	4.35
Isoleucine	6.90	4.93	4.00
Leucine	7.24	1.00	0.82
Lysine	3.83	8.04	6.92
Methionine	3.70	1.88	6.41
Methionine+Cystine	4.65	11.22	13.32
Threonine	5.20	5.19	4.32
Phenylalanine+Tyrosine	10.70	7.91	5.75
Arginine	4.50	7.16	5.81
Glycine	4.70	2.71	1.94
Histidine	2.90	11.25	10.90

SW – Silkworm meal; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal

The tested diets improved the WHC of the breast meat of SW, SWpro, and BSFd fed turkeys (13.78, 10.65, 12.90 vs. control 18.54; *P* = 0.020). In contrast, in the thigh, significant differences were recorded only for the BSFw group with 9.62 compared to control of 17.12 (*P* = 0.005). The positive trend of WHC results were reconfirmed by the cooking loss analysis in the breast meat of insect-fed turkeys (Table 3; *P* = 0.000), but no effects were obvious in the thigh portion (Table 3; *P* = 0.287). Assessment of meat color is an important factor dictated by the consumer choices. Among the three parameters – the Lightness (L*), Redness (a*) and Yellowness (b*) used for color assessment, only the Lightness (L*) was not affected by the insect diets (Table 3; *P* = 0.408, *P* = 0.104). The recordings of the Redness (a*) demonstrated response division based on insect species. The breast meat of SW and SWpro fed turkey had lower a* values of 3.54 and 2.94 compared to the control group (4.90; *P* = 0.000), while no differences were observed in BSFd and BSFw groups. In the thigh meat, however, only BSFd and BSFw diets improved the a* values with 16.47 and 16.45 respectively, while in the control this was 13.13 (*P* = 0.002). The Yellowness (b*) readings from the breast meat suggested that only BSFw diet significantly decreased the yellowness parameters (1.74 vs.control 3.79; *P* = 0.002). The SW and SWpro also had a negative effect (2.49, 2.11), although the statistical analyses did not confirm the significance. In the thigh meat, an increase of b* was recorded for turkeys fed with SWpro, BSFd, and BSFw with values of 7.16, 8.36, 7.87 respectively, compared to the control of 4.85 (*P* = 0.000).

Protein and amino acids analyses

Turkeys fed with control and treatment diets had similar (~24%) protein content among all groups in the breast meat (Table 4; *P* = 0.719) and in the thigh meat (Table 5; 19.44-20.38%; *P* = 0.508). Regarding the amino acid profiles, the breast and thigh meat responded differently, where no significant differences were observed in the breast meat (Table 4). By contrast, in the thigh meat, three amino acids responded significantly to SW and BSFw diets in comparison to the control group (Table 5). These are Valine, Isoleucine and Leucine which level increased in SW fed turkeys and decreased in BSFw group. Furthermore, the levels of these three amino acids significantly vary between BSFd and BSFw fed groups.

Cholesterol and Lipid analyses

All insect-based diets including SWpro had no effect on the cholesterol levels in both breast and thigh meat (Table 6). The new diets did not change the total lipid content in the breast (Table 6; *P* = 0.471), but differences were observed in the thigh meat. Both BSFd and BSFw-based diets increased the total lipid content in the thigh with 8.49% and 9.06% respectively, while the control had 6.16%. However, statistical significance was confirmed only for the BSFw fed group (Table 6; *P* = 0.038). To understand the effects of the insect-based diets on omega-3 and omega-6 fatty acids (FA), the levels of Eicosapentaenoic acid (EPA; n-3),

Table 2. Composition of the experimental diets

Ingredients, %	Feeding periods															
	56–78 days				79–94 days				95–114 days				115–130 days			
	C	SW	BSFd	BSFw	C	SW	BSFd	BSFw	C	SW	BSFd	BSFw	C	SW	BSFd	BSFw
Wheat	28.00	55.00	19.00	15.00	34.00	51.04	29.00	20.00	11.00	28.00	27.00	20.00	21.00	39.00	29.00	22.00
Corn	21.80	7.79	33.74	34.11	20.34	17.00	28.43	33.73	48.87	45.59	37.68	40.57	45.26	40.87	41.20	44.04
Soybean meal	33.77	16.00	21.00	24.57	28.00	11.00	15.00	19.00	23.00	6.00	8.00	12.00	17.00	–	3.00	7.00
Sunflower meal	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
SWM	–	10.00	–	–	–	10.00	–	–	–	10.00	–	–	–	10.00	–	–
BSFd	–	–	10.00	–	–	–	10.00	–	–	–	10.00	–	–	–	10.00	–
BSFw	–	–	–	10.00	–	–	–	10.00	–	–	–	10.00	–	–	–	10.00
Sunflower oil	5.00	–	5.10	5.10	6.50	–	6.50	6.30	6.60	–	7.00	7.00	6.50	–	6.80	6.80
Dicalcium phosphate	2.62	2.18	2.45	2.60	2.36	1.95	2.13	2.30	2.01	1.62	1.70	1.83	1.76	1.36	1.50	1.65
Limestone	0.55	0.92	0.58	0.61	0.51	0.86	0.61	0.61	0.40	0.75	0.58	0.62	0.43	0.77	0.55	0.58
Methionine	0.22	–	0.18	–	0.19	–	0.16	–	0.13	–	0.11	–	0.10	–	0.07	–
Lysine	0.29	0.36	0.20	0.26	0.30	0.35	0.21	0.26	0.19	0.24	0.13	0.18	0.15	0.20	0.08	0.13
Salt	0.25	0.25	0.25	0.25	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Salgard	0.20	0.20	0.20	0.20	0.20	0.20	0.40	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix,	0.20	0.20	0.20	0.20	0.20	0.20	0.16	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Optizyme	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated nutritive value																
Metabolizable energy (Kcal/kg)	2951	3045	2953	2952	3104	3120	3102	3103	3217	3216	3216	3217	3250	3253	3253	3252
Crude protein, %	22.75	22.64	22.73	22.71	20.80	20.82	20.82	20.83	18.38	18.36	18.32	18.36	16.62	16.62	16.67	16.71
Fat, %	6.84	4.06	7.08	7.84	8.30	4.28	8.36	9.02	9.00	4.87	9.07	9.87	8.86	4.81	8.97	9.77
Crude fiber, %	4.85	4.30	4.21	4.37	4.60	4.02	3.94	4.09	4.14	3.60	3.56	3.72	3.90	3.36	3.32	3.48
Phosphorus, %	0.95	0.88	0.91	0.92	0.88	0.82	0.83	0.85	0.79	0.74	0.73	0.74	0.74	0.68	0.68	0.69
Calcium, %	1.21	1.20	1.20	1.20	1.09	1.09	1.09	1.09	0.92	0.92	0.92	0.92	0.83	0.83	0.83	0.83
Lysine, %	1.50	1.51	1.50	1.51	1.36	1.37	1.36	1.36	1.11	1.11	1.10	1.10	0.92	0.92	0.93	0.93
Methionine, %	0.58	0.64	0.57	0.59	0.52	0.62	0.52	0.56	0.44	0.60	0.44	0.53	0.39	0.57	0.38	0.51
Methionine+cysteine,%	0.94	1.00	0.75	0.60	0.85	0.95	0.66	0.55	0.75	0.92	0.55	0.48	0.68	0.86	0.47	0.44
Threonine, %	0.85	1.11	0.93	0.88	0.77	1.04	0.84	0.80	0.69	0.96	0.74	0.69	0.61	0.88	0.70	0.62

SW – Silkworm meal; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal

Table 3. Effect of diets on some physicochemical properties of breast and thigh meat of turkeys

Parameters	Groups					SEM	*P*-value
	Control	SW	SWpro	BSFd	BSFw		
pH_{24h}							
BM	5.67 b	5.66 b	5.74 a	5.68 b	5.69 b	0.01	0.006
LM	5.92 a	5.70 b	5.84 a,b	6.05 a	5.78 a,b	0.03	0.001
WHC, %*							
BM	18.54 a	13.78 b	10.65 b	12.90 b	14.02 a,b	0.75	0.020
LM	17.12 a,b	12.50 a,b,c	12.83 b,c	18.02 a	9.62 c	0.85	0.005
Cooking loss,%							
BM	35.12 a	23.30 b	20.38 b	26.45 b	23.08 b	0.98	0.000
LM	31.30	32.13	29.05	37.64	31.05	1.04	0.287
Lightness, L*							
BM	54.65	54.35	54.08	53.47	53.34	0.24	0.408
LM	49.64	48.86	50.51	48.48	49.79	0.27	0.104
Redness, a*							
BM	4.90 a	3.54 b	2.94 b	3.98 a,b	3.94 a,b	0.13	0.000
LM	13.13 b	15.17 a,b	15.21 a,b	16.47 a	16.45 a	0.30	0.002
Yellowness, b*							
BM	3.79 a	2.49 a,b	2.11 a,b	3.32 a	1.74 b	0.18	0.002
LM	4.85 b	5.43 b	7.16 a	8.36 a	7.87 a	0.24	0.000

SW – Silkworm meal; SWpro – Silkworm meal + probiotic; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal; BM – breast muscle; LM – leg muscle, WHC-water holding capacity; SEM – standard error of the mean. a,b – means with different letters in the row represent significant differences at P < 0.05

Table 4. Amino acid composition of breast meat from turkeys

	Groups					SEM	*P*-value
	Control	SW	SWpro	BSFd	BSFw		
Protein, %	23.96	24.10	24.46	24.16	24.16	0.10	0.719
Amino acid, %							
Valine	0.91	1.01	0.69	0.79	0.69	0.11	0.894
Glutamine	2.08	2.08	2.68	3.00	1.92	0.53	0.974
Isoleucine	0.82	0.49	0.88	0.69	0.59	0.09	0.659
Leucine	1.64	0.97	1.75	1.36	1.17	0.17	0.661
Lysine	1.90	1.68	1.74	1.26	1.40	0.23	0.927
Methionine	0.08	0.05	0.12	0.09	0.10	0.01	0.514
Serine	0.71	0.81	0.33	0.55	0.41	0.14	0.863
Threonine	0.95	0.78	0.84	1.48	0.26	0.19	0.423
Tryptophane	0.34	0.20	0.31	0.17	0.24	0.06	0.929
Phenylalanine	0.38	0.40	0.16	0.47	1.01	0.11	0.175
Histidine	0.44	0.50	0.83	1.16	0.69	0.09	0.052

SW – Silkworm meal; SWpro – Silkworm meal + probiotic; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal; SEM – standard error of the mean

Docosahexaenoic acid (DHA; n-3), Linolenic acid (ALA; n-3), Arachidonic acid (ARA; n-6), and Linoleic (LA; n-6) were investigated in both breast and thigh meat. Like the total lipid content, the insect-based diets did not change the fatty acid levels in the breast meat, but had some effects in the thigh meat. BSFd and BSFw diets had differential effects on the FA in the thigh: BSFd fed turkeys had more EPA in the thigh (0.008%) than the control fed (0.005; P = 0.010), while the BSFw fed group had more ARA (0.010 vs. control 0.006; P = 0.047) and LA (1.78 vs. control 0.83; P = 0.018). The SW and SWpro diets impacted only ALA levels, where these were 0.120% in the thigh of the SW fed group compared to 0.010% in the control group (P = 0.004).

Mineral content

Both breast and thigh meat were subjected to mineral content analyses where the focus was on establishing the Iron, Calcium, Magnesium and Phosphorus levels (Table 7). Calcium levels were below the analytical threshold and no responses were recorded. In the breast, notable differences were recorded only for BSFw fed turkeys showing

Table 5. Amino acid composition of thigh meat from turkey

	Groups					SEM	*P*-value
	Control	SW	SWpro	BSFd	BSFw		
Protein, %	19.63	20.31	20.38	19.51	19.44	0.21	0.508
Amino acid, %							
Valine	1.01 b	1.40 a	1.17 a,b	0.97 b	0.26 c	0.11	0.000
Glutamine	4.90	1.10	2.95	0.30	4.66	0.90	0.420
Isoleucine	0.73 b,c	1.23 a	0.85 a,c	0.77 a,c	0.24 d	0.10	0.009
Leucine	1.45 b	2.46 a	1.69 a,b	1.54 a,b	0.46 c	0.20	0.009
Lysine	1.66	2.47	1.67	1.01	1.40	0.32	0.169
Methionine	0.34	0.68	0.56	0.66	0.05	0.10	0.268
Serine	0.76	0.92	0.86	0.93	0.30	0.10	0.226
Threonine	0.50	0.30	0.45	0.65	0.07	0.08	0.246
Tryptophane	0.22	0.41	0.20	0.06	0.09	0.05	0.168
Phenylalanine	1.0	1.96	1.24	1.36	0.58	0.17	0.094
Histidine	0.34	0.22	0.22	0.21	0.17	0.04	0.338

SW – Silkworm meal; SWpro – Silkworm meal + probiotic; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal; SEM – standard error of the mean

[a,b, c,d] – means with different letters in the row represent significant differences at P < 0.05

Table 6. Fatty acids composition of breast and thigh muscles

	Groups					SEM	*P*-value
	Control	SW	SWpro	BSFd	BSFw		
Breast							
Lipids, %	1.18	1.02	0.80	1.44	0.98	0.11	0.471
Cholesterol, %	<0.2	<0.2	<0.2	<0.2	<0.2	–	–
Fatty acids, %							
Eicosapentaenoic (n-3)	<0.001	<0.001	<0.001	<0.001	<0.001	–	–
Docosahexaenoic (n-3)	<0.001	<0.001	<0.001	<0.001	<0.001	–	–
Linolenic (n-3)	0.003	0.015	0.011	0.002	0.003	0.00	0.153
Arachidonic (n-6)	0.002	0.002	0.001	0.002	0.003	0.00	0.263
Linoleic (n-6)	0.157	0.118	0.083	0.170	0.143	0.01	0.372
Thigh							
Lipids, %	6.16 b,c	5.45 b	5.95 b,c	8.49 a,c	9.06 a	0.50	0.038
Cholesterol, %	0.21	0.20	0.20	0.21	0.20	0.00	0.632
Fatty acids, %							
Eicosapentaenoic(n-3)	0.005 b	0.004 b	0.003 b	0.008 a	0.005 b	0.00	0.010
Docosahexaenoic (n-3)	<0.001	<0.001	<0.001	<0.001	<0.001	–	–
Linolenic (n-3)	0.010 b	0.120 a	0.063 b	0.009 b	0.020b	0.01	0.004
Arachidonic (n-6)	0.006 b	0.005 b	0.005 b	0.006 b	0.010 a	0.00	0.047
Linoleic (n-6)	0.83 b	0.85 b	0.67 b	0.96 b	1.78 a	0.13	0.018

SW – Silkworm meal; SWpro – Silkworm meal +probiotic; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal; SEM – standard error of the mean

[a,b,c,] means with different letters in the row represent significant differences at P < 0.05

decreased levels of Magnesium (279.40 mg/kg vs. control 412.22 mg/kg), although these were over the level of statistical significance (P = 0.075). In the thigh, higher levels of Iron were noted in BSFd and BSFw fed turkeys (13.99 and 16.79 respectively) compared to the control (9.48; P = 0.029). Decrease in Magnesium was also noted in the thigh of these groups (185.26 and 174.81 vs. the control 284.82; P = 0.078).

Discussion

Overall, data suggests that insect-based diets affected the physicochemical and nutritional parameters of turkeys. While some parameters were affected differentially (eg. mineral profile) by the species-specific insect-based diets, others had no clear division on diet effects like the pH_{24}. The pH_{24} status of the breast and thigh meat responded to the new

Table 7. Minerals profile of breast and thigh meat

Minerals (mg/kg)	Groups					SEM	*P*-value
	Control	SW	SWpro	BSFd	BSFw		
Breast							
Iron	4.51	3.79	3.95	4.44	4.15	0.21	0.843
Calcium	<0.50	<0.50	<0.50	<0.50	<0.50	–	–
Magnesium	412.22	435.48	483.17	444.74	279.40	25.29	0.075
Phosphorus	2223.1	2325.7	2218.6	2160.2	2263.3	24.95	0.331
Thigh							
Iron	9.48a	9.26a	8.16a	13.99b	16.79b	1.19	0.029
Calcium	<0.50	<0.50	<0.50	<0.50	<0.50	–	–
Magnesium	284.82	224.08	268.83	185.26	174.81	18.75	0.078
Phosphorus	1886.6	1915.3	1863.3	1579.0	1552.7	58.96	0.092

SW – silkworm meal; SWpro – silkworm meal +probiotic; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal; SEM – standard error of the mean

[a,b,] means with different letters in the row represent significant differences at $P < 0.05$

diets, and although containing some statistical significance, overall, these ranges were within the normal physiological parameters (5.7-6.2; Chartrin et al., 2019). This is an agreement with results from other studies that reported either no effects (Schiavone et al., 2019) or slight decrease in meat pH from insect-fed poultry (Secci et al., 2018b; Altmann et al., 2020). The physiological significance of the pH data was confirmed by the WHC results. Significant improvements of WHC were noticeable in the breast meat of all insect-fed turkeys, where treatment groups performed with 32-42% better compared to the control (Table 3). In the thigh, however, only the BSFw diet had a positive effect on this parameter (43%; $P = 0.005$); other treatment groups had similar to the control readings of WHC. The WHC is an important index which determines the technological quality of meat, especially juiciness and meat texture (Wang et al., 2009). The muscle tissue contains about 75% water, of which only 10-15% is chemically bound. The other part is a "free water" that might be lost during cooking (Valkova-Yorgova et al. 2000). The breast meat from turkeys fed with the insect-based diets lost around ~20-26% of water during cooking, which was significantly different from this of the control 35% (Table 3; $P = 0.000$). In the thigh, however, the readings for this parameter were between ~29-37% among all groups ($P = 0.287$). The risk of development of pale, soft and exudative (PSE) condition in poultry meat is high, and one parameter indicative for the PSE status is the Lightness (L*) which has a good correlation with the pH and WHC (Jankowski et al. 2012). pH reduction in the meat results in denaturation of proteins and paler color of the meat. The differences of L* values (24h postmortem) among the control and treatment groups were not significant in both breast and thigh meat (Table 3; $P = 0.408$; $P = 0.104$), which reconfirms the technical significance of the pH_{24} results, meaning that pH_{24} responses were not significant to affect the technical parameters of the analyzed meat. Thus, data on pH_{24}, WHC, cooking loss and L*corroborate with each other leading to the conclusion that insect-based diets have the potential either to improve (particularly breast meat) meat quality or have similar effects as the control diets. This corroborates with studies carried out by Altmann et al. (2018), Pieterse et al. (2019), Schiavone et al. (2019) where analyses on breast meat of insect-fed broiler chickens did not show changes in the above parameters.

In addition to L*, the Redness (a*) and Yellowness (b*) are the other two parameters that are used for the assessment of meat color. Meat color is an important factor influencing consumer choices; therefore, effects of the new insect-based diets on meat pigmentation must be analyzed and understood. Interestingly, the current study showed response division of a*, based on insect species types of diets. The breast meat of SW and SWpro fed turkeys had significantly lower a* values of 3.54 and 2.94 compared to the control group (4.90; $P = 0.000$), while these in BSFd and BSFw groups were similar to the control. In the thigh meat, the a* index was higher in all treatment groups, but statistical significance was established only for BSFd and BSFw groups with 16.47 and 16.45 respectively, while in the control this was 13.13 (Table 3; $P = 0.002$). The new diets also affected b* index in both breast thigh meat, but with opposite trends. All recording from the breast meat of the treatment groups were lower compare to the control, but a statistical significance was established only in BSFw group (BSFw 1.74 vs. control 3.79; $P = 0.002$). An interesting fact must be noted where b* values in the breast of BSFd group was ~50% higher than BSFw ($P = 0.002$), suggesting the importance of insect meal formulations. In the thigh meat, b* was higher for turkeys fed with SWpro, BSFd, and BSFw than the control (Table 3; 7.16, 8.36, 7.87 respectively, vs. the control 4.85; $P = 0.000$). This might be influenced by the presence of carotenoids (~2.15 mg/kg) in BSF meals which has been reported previously (Secci et al. 2018a). To date, no clear effects of insect-based diets on poultry meat color are established, and this due to many factors influencing the results. First, majority of data reported responses in breast meat only, and not in thigh meat. Other factors, such as duration of feeding trials, larval feeding substrate may also contribute to the overall effects as well. In brief, it must be noted, that while Leiber et al. (2017), Altmann et al. (2018) and Pieterse et al. (2019) did not find any significant effects of insect-based diets on broilers meat color, Cullere et al. (2016) reported that a* in the breast meat of broiler quails is dependable on the dose of the insect meal, showing the highest (1.13) and lowest (0.46) values for 10% and 15%, respectively.

Dietary inclusions of insect meals had no effect on the total protein content in both breast (~24 %) and thigh (~20%) meat and were similar to the control values (Tables 4 and 5). No changes in the amino acid levels in the breast meat were observed as well (Table 4). However, in the thigh, the level of three amino acids – Valine, Isoleucine and Leucine responded to two diets in an opposite manner – a significant increase (~39-69% relative to control) in SW fed turkeys and decrease (~67-74% relative to control) in BSFw group (Table 5). Further investigations will validate the present findings. For now, it can be stated that these are branched-chain amino acids (BCAA) with significant physiological roles in vertebrates (Wu, 2013). BCAA act as building blocks for tissue proteins especially in muscles which may rich ~35% of the essential amino acids (in the muscle). In addition, BCAA have important metabolic roles (Wu, 2013), including glucose metabolism and energy processes, which might explain to some extent the increase of BCAA in the thigh of the SW fed turkeys.

In respect to total fat and cholesterol content, all insect-based diets had no effect on the breast meat (Table 6). In the thigh, however, while diets did not change cholesterol levels, some like BSFd and BSFw diets cause an increase in the fat content of the thigh. Yet, a statistical significance was established only for the BSFw group with an increase of 47% (Table 6; $P = 0.038$) compared to control. Interestingly, the thigh meat of the SW and SWpro fed turkeys did not show changes in total fat levels (compared to control), despite the high percentage of total fats in the SW meal. Poultry products are a good source of omega-3 (n-3) and omega-6 (n-6) fatty acids (FA) with a favorable n-6/n-3 ratio, particularly in turkey meat. That is why poultry meat is often regarded as a functional food. Diets can modulate FA levels in poultry meat. An example is the inclusion of flaxseeds in the turkey diet which decreased the n-6/n-3 ratio in the breast meat (Jankowski et al., 2015). To gain some understanding of how insect-based diets affect omega-3 and omega-6 FA, the levels of Eicosapentaenoic acid (EPA; 20:5(n-3)), Docosahexaenoic acid (DHA; 22:6(n-3)), Linolenic acid (ALA; 18:3 (n-3)), Arachidonic acid (ARA; 20:4(n-6)), and Linoleic (LA; 18:2 (n-6)) were investigated in both breast and thigh meat. Like the total lipid content, the insect-based diets did not change the levels of analyzed FA in the breast, but had some effects in the thigh meat. While DHA was below detectable levels for all samples, EPA levels were established, and a notable increase of 60% (compared to control) was recorded in the thigh of turkeys fed with BSFd diet. In contrast, BSFw impacted n-6 FA where the thigh meat had significantly more ARA (66%) and LA (114%). Thus, it can be concluded that the formulation type (defatted vs. whole larvae meal) of the insect meal has the potential to differentially influence the FA spectrum of the poultry meat. Results also suggest different FA responses of SW fed turkey, than those fed with BSF meals, which is expected, as each insect species has a specific FA profile that will impact poultry meat differently. However, the importance of the result is the fact that the levels of ALA were 12 times higher than those in the control (Table 6; $P = 0.004$). This corroborates with the fact that the silkworm pupae contained a high amount of ALA with reported values of 26%-36% from the total fat content (Rao, 1994; Tomotake et al., 2010). Data from previous studies also report a significant increase of ALA in meat of broilers fed with SW meal, where Mentang et al. (2013) established this in the thigh meat and Miah et al. (2020) in the breast meat. The latter study demonstrated a much lower n-6/n-3 ratio in the breast meat of SW fed broilers than the control. The n-6/n-3 ratio is a factor playing a significant role in cardiovascular diseases, and decreasing the ratio in poultry meat will ultimately improve consumers' health.

The breast and thigh meat were subjected to further analyses to measure the Iron, Calcium, Magnesium, and Phosphorus responses to the insect-based diets. Significant results were recorded only in the thigh, and not in the breast meat, where the level of Iron in BSFd and BSFw fed turkeys was 13.99 mg/kg and 16.79 mg/kg respectively vs. the control 9.48 mg/kg (Table 7; $P = 0.029$). This corroborates with the increased redness a* in the thigh of BSF fed turkeys, which can be explained by the level or status of iron-binding myoglobin. Although no statistical significance was established, the thigh meat of BSFd and BSFw fed turkeys tended to have lesser Magnesium than the control group.

Conclusions

Overall, the above result demonstrated that 10% inclusion of insect meal into turkeys' diets can modulate meat quality, with improvements of several parameters, including WHC. Breast and thigh meat responded differently to the new diets. Breast meat had significant physicochemical responses to the new diets (improved water holding capacity, cooking loss), but lacked nutritional – no change in protein, lipid, or mineral content. In contrast, in the thigh, both BSF diets increase the level of lipids and Iron. Insect diets also improve omega-3 and omega-6 fatty acid levels, and the study demonstrated that formulation types of diets (defatted vs. whole larvae meal), as well as insect species types of diets modulate FA profile differently. Inclusion of probiotics in turkeys' diet did not affect meat parameters in any specific manner. Based on the above evidence it can be concluded that inclusion of 10% insect meals in turkeys' diets does not cause detrimental effects on meat quality and is a plausible feeding strategy.

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