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Effect of Black Soldier Fly (*Hermetia illucens*) defatted flour on technological properties and quality of chicken meat

Utjecaj odmašćenog brašna crne vojničke muhe (*Hermetia illucens*) na tehnološka svojstva i kvalitetu pilećeg mesa

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ABSTRACT

Current knowledge concerning the possible use and impact of replacing the soybean proteins with those derived from a defatted black soldier fly (*Hermetia illucens*, HI) meal while feeding chickens to produce the meat for human consumption is very limited. This study's objective was to examine the influence of partial replacement of the HI-defatted flour on chicken meat's technological properties and quality. 180 one-day-old Ross 308 chicks were divided into three experimental groups (P1, P2, and P3) and one control group counting 45 chicks. The experimental groups were fed with the fattening mixtures containing 15, 25, and 45% of the HI-defatted flour, while the control group was fed in accordance with the standard chicken-fattening requirements. After 42 days, the chickens were slaughtered, and the breasts and drumsticks of 40 chickens were subjected to analysis. A statistically significant difference ($P < 0.05$) was observed in the P1 for the drip-loss values measured 24 hours after sampling, the muscle resistance to cutting, and the cooking loss values. The water-binding capacity for all three experimental groups did not demonstrate statistically significant differences for the pH₁ and pH₂ values. Considering the CIE L*a*b* color standard reference values, the results for the breast meat were normally to slightly lighter than normal ones. The basic chemical parameter (water, protein, fat, and collagen) results confirmed that there was no statistically significant difference in any of the experimental groups if compared to the control one.

Keywords: alternative protein source, broilers, production results, meat quality

SAŽETAK

Dosadašnje znanje o mogućoj upotrebi i utjecaju zamjene bjelančevina soje s bjelančevinama podrijetlom od odmašćenoga brašna crne vojničke muhe (*Hermetia illucens*, HI) u pogledu hranidbe pilića za proizvodnju mesa za ljudsku potrošnju vrlo je ograničeno. Cilj istraživanja bio je ispitati utjecaj djelomične zamjene odmašćenoga brašna HI na tehnološka svojstva i kvalitetu pilećega mesa. Sto osamdeset jednodnevnih pilića Ross 308 podijeljeno je u tri eksperimentalne skupine i jednu kontrolnu skupinu od po 45 pilića. Eksperimentalne skupine hranjene su tovnim smjesama koje su sadržavale 15, 25 i 45% odmašćenoga brašna HI, dok je kontrolna skupina hranjena u skladu sa standardnim

zahtjevima tova pilića. Svaka smjesa korištena u tovu pilića uravnotežena je prema potrebama pilića u određenoj fazi tova. Nakon 42 dana tova pilići su zaklani, a prsa i bataci sa zabatcima 40 pilića (10 po skupini) su korišteni u svrhu analiza. Statistički značajna razlika ($P < 0,05$) uočena je u P1 eksperimentalnoj skupini za vrijednosti otpuštanja mesnoga soka izmjenjenoga 24 sata nakon uzorkovanja, za otpornost mišića na presijecanje i za vrijednosti za kalo kuhanja. Kapacitet vezanja vode za sve tri pokusne skupine nije pokazao statistički značajne razlike, kao ni za vrijednosti pH_1 i pH_2 . S obzirom na referentne vrijednosti prema CIE $L^*a^*b^*$ standardu boja, rezultati za meso prsa su bili normalni do nešto svjetliji od normalnih. Rezultati analiza osnovnih kemijskih parametara (voda, bjelančevina, masti i kolagen) pokazali su kako nema statistički značajnih promjena ni kod jedne pokusne skupine u odnosu na kontrolnu skupinu.

Ključne riječi: alternativni izvori bjelančevina, brojleri, proizvodni rezultati, kakvoća mesa

INTRODUCTION

The continued global increase in chicken meat consumption is a result of several factors, including a lower price, very good availability, greater convenience, preparation ease, and an increased health implication-related consumer awareness, such as the poultry meat's lower cholesterol content, if compared to that of the red meat (Resurreccion, 2004; Michel et al., 2011; Haley, 2015). The International Agency for Research on Cancer (IARC) recently conducted a meta-analysis of 800 epidemiological studies and found that at least half of them indicate an increased colorectal-cancer risk among red meat consumers, especially bovine, porcine, and other originating from large livestock (WHO, 2015). On the other hand, in the last decade, the use of insects as a source of energy, food, and feed has brought significant benefits to the environment, economy, and the provision of sufficient food in the world. Through their activity, the insects can convert low-value biomass into nutritionally valuable proteins, thus contributing to the preservation of the environment and better efficiency of the utilization of food-industry and agriculture waste as secondary raw materials for feed production (van der Spiegel et al., 2013; Allegretti et al., 2018).

Poultry feeding is one of the most important factors in poultry production technology. It is known that the costs associated with feeding poultry account for 65-75% of the total costs of poultry meat production (Kralik et al., 2008). In the standard broiler fattening, mainly the soybean proteins were used to a much lesser extent, or very rarely, than other feedstuffs (peas, broad beans, and alfalfa meal). The essential amino acids (methionine

and tryptophan) are compensated in a synthetic form because they are very expensive and increase the price of the feed considerably. Research heretofore demonstrates that it is necessary to find a substitute for the protein component in the chicken feed that is economically viable and ecologically acceptable (FAO, 2009; FAO 2014). For these reasons, there is a necessity for an additional protein source pertaining to the fattening of animals from alternative sources, including the insect rearing (van der Spiegel et al, 2013; Cullere et al, 2016; Wang and Shelomi, 2017; Cullere et al, 2018; Allegretti et al, 2018; Caligiani et al, 2018).

The results of the studies carried out to date, in which the insect meal was used as a partial or complete replacement of the protein component in a standard diet, differ considerably (Makinde, 2015). Leiber et al. (2017) found that the broiler diets containing crude protein from *Hermetia illucens* larvae resulted in similar or better conversion efficiency results when compared to the soybean protein and that a partial replacement of the soybean cake with various combinations of alfalfa protein or *Hermetia illucens* protein did not affect the growth performance of broilers when compared to the standard poultry diets. De Marco et al. (2015) conducted a study on a nutritional value of diets derived from the insect meal of *Tenebrio molitor* and *Hermetia illucens* used for the feeding of broilers and circumstantiated that both types of diets are a valuable source of easily digestible amino acids and metabolic energy, especially in the case of a meal derived from *Tenebrio molitor*. The larvae of the *Hermetia illucens* fly are considered a good alternative in

poultry feeding, as they have a very favorable amino acid composition, with a high lysine and methionine content (Leiber et al., 2017).

For all these reasons, this study aimed to investigate the influence of a partial replacement of defatted *Hermetia illucens* meal on chicken meat's technological characteristics and quality. It is expected that this research will lead to new insights into the most appropriate effects of feeding poultry with a black soldier fly meal with regard to the chicken meat quality. The data obtained will contribute to an informed consideration of the possibility of using this protein source in a chicken diet as a food source for human consumption.

MATERIAL AND METHODS

For this study, a total of 180 Ross 308 one-day-old chickens were used for fattening. The chickens were first weighed and housed in pens adapted to the needs of the experiment. For each experimental group, forty-five broiler chickens were separated and then divided into three subgroups of fifteen chickens each. For feeding, the mixtures that met the broiler-related nutritional requirements in all parameters were put together. Each mixture used in fattening was tailored to the needs of the chickens at a specific stage of fattening. The compositions of the mixtures are listed in Tables 1–3, as are the chemical compositions of individual feed types (starter, grower, and finisher) for the control group (K) and the experimental groups (P1, P2, and P3). In experimental group 1 (P1), 15% of the soybean protein was replaced by the HI protein; in experimental group 2 (P2), 25% of the soybean protein was replaced by the HI protein; and in experimental group 3 (P3), 40% of the soy protein was replaced by the HI protein, using the defatted HI larvae meal. The broilers' weight, feed intake, and health status were monitored in all production cycles.

All animals used in the research were slaughtered in an approved abattoir in accordance with the Council Regulation (EC) No. 1099/2009 of 24 September 2009 on the protection of animals at the time of slaughtering and in compliance with all professional rules.

Technological meat parameters

The technological chicken breast-meat parameters encompassed the determination of pH₁ and pH₂ values, drip loss, water binding capacity (*Sp.v.v.*), muscle resistance to cutting, cooking loss, and the meat color.

pH₁ and pH₂ values

The meat-related pH and temperature were measured in all samples of *Pectoralis superficialis* muscle within 45 minutes post-slaughter (pH₁ and T1) and 24 hours post-slaughter and after refrigerating the meat to the temperature of +4 °C (pH₂ and T2). The pH and temperature values were measured by a digital pH meter (Mettler MP120-B, Zurich, Switzerland) by inserting the pH meter probe into the left half of the pectoral muscle. The pH drumstick value was measured in the same way.

Drip loss

The drip loss was determined using the bag method according to Honikel (1998). In addition to the abovementioned method, a drip loss of the pectoral muscle was also determined using the EZ drip-loss method (Danish Meat Research Institute (DMRI), 2010) for comparison purposes. The samples were taken from the thickest part of the pectoral muscle (sample characteristics: 3 cm high and 2 cm in diameter). To determine the drip loss using the Honikel bag method, the weighed samples were placed in a PVC bag and stored in a refrigerator at +4 °C. After 24 hours, the samples were weighed again. After 48 hours, the samples were weighed yet another time, and the drip-loss value was calculated by applying the following formula:

$$OMS = (PV - PZ) / PV \times 100,$$

where the OMS is a drip loss (%), PV is an initial tissue weight (g), and the PZ is the final tissue weight (g).

When determining the drip loss using the EZ drip-loss method, each individually weighed sample was placed in a special plastic meat container, stored in a refrigerator at a temperature of +4 °C, and weighed after 24 and 48 hours, respectively.

Table 1. The composition of starter feed mixtures used for broiler fattening (%)

COMPONENT	K	P1	P2	P3
Corn	54.76	58.86	61.85	60.17
Wheat fodder flour	-	-	-	5.5
Soybean meal (44.5%)	38	30	24.5	15.4
Threonine	0.15	0.18	0.18	0.21
Salt	0.21	0.21	0.21	0.21
Fodder chalk	1.6	1.6	1.6	1.6
MCP	1.3	1.3	1.3	1.1
Soybean oil	2.6	1.1		
Methionine liquid	0.28	0.28	0.28	0.3
Lysine sulfate	0.45	0.58	0.7	0.9
Baking soda	0.15	0.15	0.15	0.15
ptp-monesin	0.5	0.5	0.5	0.5
Flour BSF (63,02%)	-	5.24	8.73	13.96
TOTAL (%)	100	100	100	100
CHEMICAL COMPOSITION				
Protein	21.99	22.02	21.99	22
Fat	5.01	4.1	3.4	3.95
Fibers	2.88	3.16	3.34	4.13
Moisture	11.71	11.79	11.85	11.89
ME MJ/kg	12.56	12.58	12.59	12.64
Ca	0.97	0.98	0.98	0.95
P	0.67	0.69	0.7	0.7
P usable	0.42	0.41	0.4	0.34
Na	0.16	0.16	0.16	0.15
Lysine	1.44	1.42	1.42	1.43
Methionine	0.56	0.56	0.56	0.57
Threonine	0.95	0.96	0.94	0.93
Poultry MJ/SP	0.57	0.57	0.57	0.57
Ash	6.72	6.83	6.9	7.14

BSF – black soldier fly; K – initial mixture for the control group; P1 – initial mixture for experimental group 1 (proportion of proteins originating from BSF larvae 15%); P2 – initial mixture for experimental group 2 (proportion of proteins originating from BSF larvae 25%) P3 – initial mixture for experimental group 3 (proportion of proteins originating from BSF larvae 40%); MCP – monocalcium phosphate; ptp-monesin – coccidiostat.

Table 2. The composition of grower feed mixtures used for broiler fattening (%)

COMPONENT	K	P1	P2	P3
Corn	56.89	46.7	49.36	52.61
Feed wheat	-	10	10	10
Wheat fodder flour	-	4	4.5	5
Soybean meal (44,5%)	36	27	21.5	13.8
Threonine	0.12	0.12	0.12	0.12
Salt	0.21	0.21	0.21	0.21
Fodder chalk	1.3	1.3	1.3	1.3
MCP	1.3	1.3	1.2	1.3
Soybean oil	3	3	2	0.7
Methionine liquid	0.23	0.23	0.23	0.23
Lysine sulfate	0.3	0.47	0.57	0.7
Baking soda	0.15	0.15	0.15	0.15
ptp-monesin	0.5	0.5	0.5	0.5
Flour BSF (63,02%)	-	5.02	8.36	13.38
TOTAL (%)	100	100	100	100
CHEMICAL COMPOSITION				
Protein	21.07	21.18	21.09	21.12
Fat	5.45	5.78	5.16	4.42
Fibers	2.84	3.47	3.69	4
Moisture	11.69	11.59	11.67	11.73
ME MJ/kg	12.77	12.77	12.77	12.79
Ca	0.85	0.85	0.84	0.87
P	0.67	0.7	0.69	0.73
P usable	0.42	0.41	0.37	0.38
Na	0.16	0.16	0.16	0.15
Lysine	1.3	1.3	1.29	1.28
Methionine	0.51	0.5	0.5	0.5
Threonine	0.89	0.86	0.84	0.82
Poultry MJ/SP	0.61	0.6	0.61	0.61
Ash	6.28	6.65	6.62	6.85

BSF – black soldier fly; K – growth mixture for the control group; P1 – growth mixture for experimental group 1 (proportion of proteins originating from BSF larvae 15%); P2 – growth mixture for experimental group 2 (proportion of proteins originating from BSF larvae 25%) P3 – growth mixture for experimental group 3 (proportion of proteins originating from BSF larvae 40%); MCP – monocalcium phosphate; ptp-monesin – coccidiostat.

Table 3. The composition of finisher feed mixtures used for broiler fattening (%)

COMPONENT	K	P1	P2	P3
Corn	61.03	64.47	66.87	61.36
Feed wheat	-	-	-	10
Soybean meal 44,5%	31	24.3	19.8	12
Threonine	0.12	0.12	0.12	0.12
Salt	0.21	0.21	0.21	0.21
Fodder chalk	1.3	1.3	1.3	1.3
Captex	0.1	0.1	0.1	0.1
MCP	1.1	1.1	1.1	1.1
Soybean oil	4	2.7	1.8	0.5
Methionine liquid	0.21	0.23	0.21	0.22
Lysine sulfate	0.28	0.4	0.47	0.65
Baking soda	0.15	0.15	0.15	0.15
Pt - Z	0.5	0.5	0.5	0.5
Flour BSF 63,02%	-	4.42	7.37	11.79
TOTAL (%)	100	100	100	100
CHEMICAL COMPOSITION				
Protein	19.04	19.09	19.09	19.12
Fat	6.5	5.69	5.12	4.13
Fibers	2.7	2.93	3.09	3.31
Moisture	11.61	11.7	11.73	11.72
ME MJ/kg	13.22	13.24	13.25	13.19
Ca	0.80	0.81	0.81	0.82
P	0.60	0.62	0.63	0.64
P usable	0.37	0.35	0.34	0.33
Na	0.16	0.16	0.16	0.16
Lysine	1.16	1.15	1.14	1.16
Methionine	0.47	0.48	0.46	0.47
Threonine	0.82	0.8	0.79	0.76
Poultry MJ/SP	0.69	0.69	0.69	0.69
Ash	6	6.05	6.1	6.23

BSF – black soldier fly; K – finisher mixture for the control group; P1 – finisher mixture for experimental group 1 (proportion of proteins originating from BSF larvae 15%); P2 – final mixture for experimental group 2 (proportion of proteins originating from BSF larvae 25%) P3 – final mixture for experimental group 3 (proportion of proteins originating from BSF larvae 40%); Captex – the commercial name of an enzymatic agent for breaking down mycotoxins in feed; MCP – monocalcium phosphate; Pt-Z – vitamin-mineral premix, without coccidiostats.

After weighing the container with the meat sample and the meat juice, the meat sample was removed by the tweezers, and only the container with the meat juice was weighed. The drip loss (%) was calculated after 24 and 48 hours by applying the following formula:

$$EZ \text{ DrippLoss} = ((Wl - Wc)) / ((Wt - Wc)) \times 100,$$

where *Wl* is a container mass containing meat juice (g), the *Wc* is an empty container mass (g), and the *Wt* is a container mass containing meat with the meat juice (g).

Water-binding capacity

Water-binding capacity (*Sp.v.v.*), a compression method according to Graü-Hamm (1952), was performed by cutting 0.3 ± 0.01 g of muscle tissue and compressing it for five minutes with compression goggles for trichineloscopy on filter paper. The *Sp.v.v.* value (expressed in cm²) was obtained by determining the surface area wetted by the squeezed juice using a digital planimeter 350 E HAFF (HAFF GmbH Feinmechanik, Pironten, Germany).

Muscle resistance to cutting

On forty samples (ten samples per test group), the muscle cutting resistance was determined using a Warner-Bratzler knife connected to the TA.XTplus Texture Analyzer device (Stable Micro Systems, Surrey, UK). The cutting resistance was measured on the left half of the thawed pectoral muscle after it had been frozen and stored at -20 °C for 21 days (Liu et al., 2004). The maximum force required to cut the pectoral muscle sample (WBSF (N)) was determined using the *Texture Exponent 4.0* program from Stable Microsystems (Stable Microsystems, Surrey, UK).

Cooking loss

The cooking loss was determined on the breast muscle-tissue samples, whereby the cooking loss is also included in the thawing calo and calculated according to the following formula:

$$KK = \frac{m(1) - m(2)}{m(1)} \times 100$$

where the *KK* is a cooking loss (%), *m(1)* is the wight of the sample before cooking, and *m(2)* is the weight of the sample after the cooking.

Meat color

The color of skinless meat was determined 24 hours after slaughter and chilling using a Minolta CR-300 portable colorimeter (Minolta Camera Co. Ltd., Osaka, Japan). The values were determined according to the CIE L*a*b* system: L* (degree of lightness), a* (degree of redness) and b* (degree of yellowing).

Chemical parameters of meat

The chemical parameters of chicken meat were determined using a validated standard and in-house analytical methods. The methods included the determination of water and ash content, total fat, total protein, and collagen.

All chemicals used for the determination were of an analytical grade. All samples were homogenized in a homogenizer (*Grindomix GM 200*, Retch, Haan, Germany) for 15 seconds at 6,000 rpm and stored in plastic containers filled to the brim to slow down the spoilage processes.

The water content was determined gravimetrically according to the ISO method (ISO 1442: 1997) using a thermostat (*Epsa 2000*, Ba-Ri, Velika Gorica, Croatia) and drying at 103 °C. The ash content was determined according to the ISO method (ISO 936: 1998) by incineration in a muffle furnace at 550 °C (LV9 / 11 / P320, Nabertherm, Lilienthal, Germany). Total lipids were determined according to the Soxhlet method (HRN ISO 1443: 1999), whereby the lipids were extracted by the ether of the extraction devices (Soxtherm 2000, Gerhardt, Koenigswinter, Germany). Total proteins were determined according to the Kjeldahl method (HRN ISO 937: 1999) using a destruction unit (Unit 8 Basic, Foss, Höganäs, Sweden) and a distillation and titration device (Kjeltec 8400, Foss, Höganäs, Sweden). The collagen content was determined according to the HRN ISO 3496:1999 method, which describes the spectrophotometric determination of hydroxyproline (DR/4000U, Hach, Duesseldorf, Germany).

Statistical analysis

A statistical analysis regarding the results obtained was carried out using the SAS computer program (SAS Institute Inc., Cary, New Carolina, USA, 2000).

The differences between the feeding treatments in the production-indicator results and the technological and chemical characteristics of chicken meat were subjected to an analysis of variance using the PROC GLM procedure in the SAS program. The differences' significance was tested using the post hoc LSD and Tuckey's test at the significance level (P) of 0.05.

The chemical parameter results were compared to the experimental groups (control, P1, P2, and P3) and the meat types (breast muscle and drumstick).

RESULTS AND DISCUSSION

After slaughter, the technological quality indicators of the pectoral muscle and drumsticks were measured. In the experimental group P1, a statistically significant difference ($P < 0.05$) was detected in the values for drip loss measured 24 hours after sampling, a drip loss, cooking loss, and muscle resistance to cutting, as shown in Table 4. The results for the drip-loss indicator, measured 24 and 48 hours after slaughter, comply with the experimental values obtained by Kralik et al. (2013) for these hybrids.

It is known that muscles contain about 75% of water, with a part thereof being firmly bound to muscle proteins and a part being present as immobilized water in the muscle. The bound part of the water is smaller and is not affected by changes such as heat treatment or the cessation of *rigor mortis*, whereas the immobilized water is affected by these changes. As a result of the changes in the structure of the muscle cell—that is, the transformation of muscle into meat after slaughter—lactic acid accumulates in the tissue, which leads to a drop in the pH value of the meat. Subsequently, the obtained pH values measured after 24 hours are lower than those obtained by measuring after 48 hours, in accordance with the described biochemical processes. The muscle pH primarily refers to the biochemical state of the muscle at the time of slaughter and the development of *rigor mortis*.

Fletcher (2002) points out that the muscle pH affects the ability to bind water from the protein and therefore has a direct effect on the physical structure of the meat and its characteristics (Briskey, 1964). In general, muscle pH is a key factor in meat quality and is also reflected in other technological indicators. The meat with pH values close to the isoelectric point (5.2–5.5) of the proteins of which it is composed has lower values concerning a water-binding capacity and higher values concerning cooking loss. Allen et al. (1998) also detected that the low pH value of poultry meat is associated with a low water-binding capacity, resulting in an increased cooking time and a higher drip loss. The research results for drip loss, cooking time, and muscle resistance to cutting obtained in this study can only be compared with the results obtained by Cullere et al. (2016), which relate to the effects of a change in dietary regime due to the partial replacement of the protein component of a plant origin by the protein of an animal origin. Their studies circumstantiated that the breast meat of Japanese quail fed with a partial replacement of the protein component derived from a defatted black soldier fly meal, at 15% in the feed mixture, had a lower pH value (5.67) being closer to the isoelectric point, resulting in lower water-binding capacity values—that is, in the higher cooking-loss values. Nevertheless, the final quality of the meat did not indicate any changes in the muscle resistance to cutting, which means that this does not affect the tenderness of the meat.

The results of water-binding capacity for all three experimental groups, as well as the drip loss after 48 h, have indicated no statistically significant differences when compared to the control group (Table 4). In previous studies, it was detected that the water-binding capacity can be influenced by animal feeding (Karolyi, 2004; Žilić et al.; 2016). The results obtained therefore suggest that the partial replacement of soybean proteins with the proteins from the defatted meal of *Hermetia illucens* larvae had no negative effect on the water-binding capacity and the drip loss of chicken meat. A statistically significant difference was detected in the value of the cooking-loss indicator for the experimental group P1, which had a significantly lower value when compared to the control group and the

experimental group P3. If the change in the composition of the feed mixture is considered, it can be seen that this indicator had a significant influence on the composition of the muscle tissue. This change can also be seen in an indicator of the muscle resistance to cutting, which is also statistically significantly different from that of the control group, indicating that the meat of experimental group P1 was more tender. This result is not consistent with those of the study by Cullere et al. (2016), who detected that the cooking-loss values in the experimental group fed with a replacement consisting of 15% of the defatted *Hermetia illucens* larvae meal in the feed mixture were statistically significantly higher than those in the experimental group fed with a replacement consisting of 10% of the defatted *Hermetia illucens* larvae meal in the feed mixture.

Previous studies have proven that poultry meat with lower pH values is less tender and has a higher resistance to cutting (Froning et al., 1978; Barbut, 1993). In this study, the pH of the breast muscle and drumsticks ranged from 5.96 to 6.06 for all three test groups (Table 5), with no statistically significant difference in pH₁ and pH₂ values between the tested groups for either breast muscles or the drumsticks. On the other hand, the results for muscle resistance to cutting demonstrate a statistically significant difference for the experimental groups P1 and P3, which are lower if compared to the values of the control group. It was detected that the meat in the experimental groups had a lower muscle resistance to

cutting—that is, it was more tender. In the experimental group P2, the value of this indicator is also lower than the value of the control group ($P > 0.05$). It can therefore be concluded that the replacement of soybean proteins with proteins originating from *Hermetia illucens* has a positive effect on the tenderness of the meat with regard to this technological indicator.

The pH₂ values determined for the breast muscles and drumsticks are consistent with the results described in the literature for the hybrid Ross 308 (Qiao et al., 2002; Honikel et al.; 1981; Davis et al., 1974). Cullere et al. (2016) confirmed that the pH values of the meat in the experimental groups of the Japanese quail, if compared to the control group, were statistically significant ($P < 0.001$) when introduced as a partial replacement of the protein component derived from the defatted black soldier fly meal in proportions of 10 and 15% but within the range of normal pH values for the Japanese quail meat. In a more recent study, Cullere et al. (2019) replaced the soybean oil with a fat originating from the black soldier fly larvae in the proportions of 50 and 100%, whereby the pH values of the breast muscles were not statistically significant and amounted to 6.32 and 6.30, respectively, while the drumstick pH value was 6.41 in both experimental groups. In this study, the pH results of the chicken meat indicate that the partial replacement of soybean protein with the protein originating from *Hermetia illucens* did not negatively affect the pH value of the fresh chicken meat.

Table 4. Technological quality indicators for breast muscles

Test group	K	P1	P2	P3	P value
Statistical parameter	$\bar{x} \pm \text{sd}$	$\bar{x} \pm \text{sd}$	$\bar{x} \pm \text{sd}$	$\bar{x} \pm \text{sd}$	
Drip loss after 24 h (%)	1.17 ± 0.03 ^b	1.65 ± 0.19 ^a	1.16 ± 0.24 ^b	1.39 ± 0.33 ^{ab}	0.029
Drip loss after 48 h (%)	3.23 ± 0.72	2.62 ± 0.68	2.64 ± 0.79	3.17 ± 1.13	0.636
Sp.v.v. (cm ²)	6.70 ± 0.91	6.91 ± 0.79	7.19 ± 1.49	6.24 ± 0.65	0.582
Cooking loss (%)	24.91 ± 1.16 ^a	19.90 ± 1.49 ^b	23.83 ± 2.16 ^a	23.57 ± 2.24 ^{ab}	0.014
Muscle resistance to cutting (N)	38.95 ± 2.19 ^a	31.49 ± 1.03 ^b	35.49 ± 4.90 ^{ab}	29.26 ± 2.98 ^b	0.006

\bar{x} - mean value; sd - standard deviation; values in the same row marked with different letters (a, b) are statistically different according to the Tukey test ($P < 0.05$).

Table 5. The pH values for the breast muscles and drumsticks

Test group	K	P1	P2	P3	P value
Statistical parameter	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	
pH ₁ breast muscle	6.34 ± 0.06 ^b	6.49 ± 0.06 ^a	6.42 ± 0.04 ^{ab}	6.35 ± 0.03 ^b	0.021
pH ₂ breast muscle	5.94 ± 0.07	6.00 ± 0.06	5.96 ± 0.06	6.06 ± 0.06	0.159
pH ₁ drumsticks	6.51 ± 0.07	6.60 ± 0.11	6.56 ± 0.02	6.49 ± 0.06	0.228
pH ₂ drumsticks	6.31 ± 0.09	6.25 ± 0.06	6.21 ± 0.05	6.20 ± 0.04	0.298

\bar{x} - mean value; sd - standard deviation; values in the same row marked with different letters (a, b) are statistically different according to the Tukey test ($P < 0.05$)

When interpreting the previously described indicators, it is necessary to connect them to the values relating to the breast muscles and the drumstick color (Table 6). In the case of the breast muscles, statistically significant differences ($P < 0.05$) were demonstrated by the values of L* in the P3 experimental group, which is lower than the value in the control group, and the values for b* in P2 and P3 of the experimental group. The values for a* did not demonstrate a statistically significant difference in any test group ($P < 0.05$). Similarly, no statistically significant difference was detected with regard to any measured color-property value concerning the drumsticks in all test groups.

Considering the reference values according to the CIE L*a*b* color standard, the results of this muscle tissue examination can be described as normal to

slightly lighter than normal. Since the breast-meat and drumstick pH values are approximately 6.00 (Table 5), it can be concluded that the results of the color values are in agreement with the previous studies (Barbut, 1993; Boulianne and King, 1995 and 1998; Allen et al., 1997; Fletcher, 1999; Fletcher et al., 2000).

A higher pH value is associated with the darker meat (drumsticks), while a lower pH value is associated with the lighter meat (breasts). Both values indicate the changes in the functional properties of meat or are considered to be the factors contributing to the variations in the product. Le Bihan-Duval et al. (1999) indicated that the chicken breast meat had an average pH value of 5.77 and an average L* value of 50.7. Lonergan et al. (2003) measured an average pH value of 5.82 and an average L* value of 43.34 for the chicken breast meat. Kralik et al.

Table 6. The average values of breast muscle and drumstick color

Test group		K	P1	P2	P3	P value
Statistical parameter		$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	
Breast	CIE L*	55.63 ± 1.04 ^a	53.89 ± 1.12 ^{ab}	55.31 ± 1.38 ^a	52.48 ± 1.22 ^b	0.039
	CIE b*	2.38 ± 0.26	2.08 ± 0.53	1.95 ± 0.29	2.21 ± 0.23	0.336
	CIE a*	4.64 ± 0.79 ^b	5.91 ± 0.49 ^{ab}	6.77 ± 0.90 ^a	6.25 ± 0.79 ^a	0.043
Drumsticks	CIE L*	54.07 ± 2.19	50.98 ± 1.74	53.29 ± 2.26	52.89 ± 1.96	0.280
	CIE b*	12.08 ± 0.71	12.08 ± 1.25	12.91 ± 1.85	13.36 ± 0.38	0.096
	CIE a*	8.58 ± 0.97	7.50 ± 0.45	8.64 ± 1.47	8.90 ± 1.54	0.207

\bar{x} - mean value; sd - standard deviation; values in the same row marked with different letters (a, b) are statistically different according to the Tukey test ($P < 0.05$)

(2006) examined the color and the pH value of chicken meat intended for grilling originating from the domestic market and detected that the pH values of breast-muscle meat ranged from 5.92 to 6.16, with the L^* value ranging from 50.88 to 54.34, while the pH value for the dark meat ranged from 6.05 to 6.36. The values for L^* ranged from 51.97 to 52.79. According to the CIE standard $L^*a^*b^*$, the values for a^* and b^* range from -120 to +120, with the a^* value ranging from green if negative to red if positive, while the b^* value ranges from blue if negative to yellow if positive (Papadakis et al., 2000; Yam and Papadakis, 2004).

In the more recent studies related to a modification of the feed mixture by the introduction of proteins from *Hermetia illucens*, Cullere et al. (2016) indicated that the total pH value measured in the breast muscle was lowest (5.67) with a protein content of 15% derived from the defatted meal of *Hermetia illucens* larvae. The values for a^* were statistically significant when compared to the control group, with the lowest value of 0.46, while the values for L^* were consistent and ranged from 54.4 to 55.1. Since previous studies have established that muscle color can be influenced by diet—that is, by the composition of the mixture (Mugler et al, 1972; Allen et al, 1978; Smith et al, 2002; Qiao et al, 2002; Cullere et al, 2016)—the results obtained in this study suggest that the partial replacement of soybean proteins with proteins derived from the defatted meal of *Hermetia illucens* larvae did not have a negative effect on the meat color.

To evaluate the effects of replacing a part of the proteins of plant origin with the proteins originating from the defatted meal of *Hermetia illucens* larvae on the chemical properties of chicken meat (water content, protein content, fat content, ash and collagen content), the breast muscle-meat and drumstick analyses were performed subsequent the end of the fattening period.

Concerning the chicken breast meat (Table 7), a statistically significant difference ($P < 0.05$) was detected with regard to the water content between the test groups P1 and P2 and with regard to the protein content between the test groups P1 and P2, as well as with regard to the P1 and P3 group. However, no statistically significant

difference was detected between the control group and the experimental group P1. No statistically significant difference was detected concerning fat content, ash, and collagen in any of the test groups if compared to the control group.

With regard to the drumsticks (Table 8), a statistically significant difference ($P < 0.05$) was detected concerning the proportion of protein in the experimental group P1 if compared to the control group, and the two other experimental groups—P2 and P3—as well as for the proportion of ash concerning the P2 and P3 of the experimental groups.

Previous research on the quality of chicken meat has indicated that chemical composition is influenced by genetic predisposition, hybrids, the way chickens are kept or reared, feeding, and short-term, post-mortem stress (Fremery, 1966; Acton, 1973; Evans et al, 1976; Cunningham et al, 1977; Farrell, 1991; Smith et al, 1993; Xiong et al, 1993; Qiao et al, 2002). Xiong et al. (1993) indicated that, in various hybrids, the breast-meat protein content fluctuated between 20.7% and 23.6%, the water content fluctuated between 74.6% and 75.9%, and the fat content fluctuated between 1% and 2%. In chicken drumsticks, the values are slightly different, and the protein content varies between 18.1% and 21.3%, the water content between 72.8% and 73.8%, and the fat content between 5.0% and 7.2%. By comparing the data from this research with the available literature data, it can be seen that the changes in the composition of the mixture did not lead to significant deviations in the experimental groups fed with different proportions of *Hermetia illucens* in terms of the values of water, protein, collagen, and fat content, breast meat, or drumsticks in relation to the quality indicators described in the previous research.

The breast-meat and drumstick quality of conventionally fattened Ross 308 hybrid chickens was investigated by Kralik et al. (2001). They indicated that the average breast meat water content amounted to 74.01%, the protein content amounted to 24.15%, the fat content amounted to 0.62%, and the ash content amounted to 1.22%.

Table 7. Chemical properties of chicken breast-muscle meat (%)

Test group	K	P1	P2	P3	P value
Statistical parameter	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	
Water	75.93 ± 0.17 ^{ab}	76.33 ± 0.47 ^a	75.04 ± 0.63 ^b	75.77 ± 0.15 ^{ab}	0.007
Total protein	19.84 ± 0.53 ^a	20.15 ± 0.44 ^a	21.49 ± 0.05 ^b	21.41 ± 0.16 ^b	0.001
Total fat	2.59 ± 0.37	2.11 ± 0.11	2.23 ± 0.61	2.13 ± 0.28	0.168
Ash	1.16 ± 0.03	1.12 ± 0.01	1.19 ± 0.04	1.20 ± 0.05	0.129
Collagen	0.54 ± 0.09	0.47 ± 0.04	0.46 ± 0.01	0.51 ± 0.04	0.350

\bar{x} - mean value; sd - standard deviation; values in the same row marked with different letters (a, b) are statistically different according to the Tukey test ($P < 0.05$)

Table 8. Chemical properties of chicken drumsticks (%)

Test group	K	P1	P2	P3	P value
Statistical parameter	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	
Water	74.59 ± 0.45	75.63 ± 0.47	74.26 ± 0.44	74.54 ± 0.24	0.239
Total protein	18.89 ± 0.21 ^a	17.22 ± 0.02 ^b	18.73 ± 0.43 ^a	18.09 ± 0.31 ^a	0.001
Total fat	6.69 ± 0.91	5.36 ± 0.71	7.08 ± 0.42	6.64 ± 0.35	0.291
Ash	1.02 ± 0.02 ^{ab}	1.01 ± 0.00 ^{ab}	0.98 ± 0.01 ^b	1.04 ± 0.01 ^a	0.013
Collagen	1.09 ± 0.15	1.30 ± 0.03	0.17 ± 0.17	1.26 ± 0.10	0.211

\bar{x} - mean value; sd - standard deviation; values in the same row marked with different letters (a, b) are statistically different according to the Tukey test ($P < 0.05$)

The chemical parameter values concerning the drumsticks were slightly different so that the average water content amounted to 74.56%, the protein content amounted to 20.96%, the fat content amounted to 3.29%, and the ash content amounted to 1.19%.

The previously figured values are different from the values obtained in this study, but it is important to emphasize that the authors fattened the chickens with only two fattening mixtures: the starter mixture, which contained 21.6% protein, and wherewith the chickens were fed from the 1st to the 28th day of the fattening period, and the finisher mixture, which contained 18.3% protein when the chickens were fed from 29th to the 42nd day of the fattening period. This resulted in a higher proportion of protein and a lower proportion of fat in the chicken meat. This type of fattening differs from

the manufacturer's recommendations for these hybrids, but this was carried out to test the possibility of the production of chickens with a higher protein and a lower fat content. Once again, the results obtained confirm that the feeding has a significant influence on the chicken meat's composition and quality.

Xiong et al. (1992) investigated the differences in the chemical composition of breast meat and drumsticks of eight different genetic lines from commercial breeding, which had also been fed with commercial mixtures based on corn and soybean and had been fattened for eight weeks. The protein content in the breast meat varied between 20.7% and 23.6%, the water content between 74.6% and 75.9%, and the fat content between 1% and 2%. For the drumsticks, these values differed, so that the protein content was lower, from 18.1% to 21.3%. The

water content was also lower, from 72.8% to 73.8%, but the fat content was higher, from 5% to 7.2%.

In this study, the chemical parameter results concerning the protein content and drumstick fat content (Table 8) comply with the results of Xiong et al. (1992). The breast meat values concerning the protein content and water content did not differ significantly. Also, the slightly higher values with regard to the breast-meat fat content (Table 7) can be interpreted as slightly higher. However, significant differences in the chemical quality parameters between the results obtained by Xiong et al. (1992) and the results of this study were generally not observed despite the partial replacement of soybean proteins by *Hermetia illucens* proteins.

Cullere et al. (2018) investigated the influence of a partial replacement of soybean proteins with a defatted *Hermetia illucens* flour in the proportions of 10% and 15% on the chemical parameters of partridge meat and detected that there was no significant influence or change in the observed parameters, either if compared to the control group or the experimental groups. Other researchers (Loponte et al., 2017; Dabbou et al., 2018; Gariglio et al., 2019), who investigated the effect of replacing the soybean proteins with the *Hermetia illucens* ones, did not carry out tests concerning the indicators that determine the chemical quality of the meat, which in this respect provides for a comparison with the results of this study impossible.

However, the values that define the chemical properties of chicken meat can be related to certain technological parameters such as the meat color and pH value to determine the differences in meat quality—that is, to determine the meat quality earmarked for further processing. Qiao et al. (2002) investigated this correlation with regard to the breast muscles obtained from three different producers. They found out that the protein content was significantly lower in the breast meat (22.44%) than in the drumsticks (22.96% and 23.27%), but there was no significant difference in fat and ash content. The average pH values for normal or lighter-than-normal meat were between 5.95 and 5.82, while they amounted

to 6.23 for darker meat. The L^* values for normal—that is, for the lighter-than-normal—as well as for the dark meat, were consistent with the values described in the previous studies by the same authors (Qiao et al., 2002). Similar results were described by Flecher et al. (2000). In this study, the protein content values obtained in all three experimental groups, indicate that the breast-muscle meat can be classified as light if compared to the results of the study by Qiao et al. (2002), which is consistent with the pH value and the L^* color values. The comparison of the results with this study's control group and with the other authors' results allows for a conclusion that the partial replacement of soybean proteins by the *Hermetia illucens* ones did not exert a negative influence on the chemical quality parameters of chicken meat in any of the experimental groups.

CONCLUSION

Based on the results obtained concerning the technological properties and chemical quality of chicken meat, the present study has provided new data and insights on the potential use of partial substitution of soybean proteins with a defatted meal obtained from *Hermetia illucens* as a new sustainable feed.

The main results indicate that there were no statistically significant differences in the chemical characteristics of chicken meat, which demonstrated good technological properties, especially in terms of the meat color and tenderness as the most important parameters when it comes to the consumer's decision to purchase and prepare the meat for consumption.

The results obtained for the water-binding capacity and chicken meat drip loss suggest that a partial replacement of soybean proteins with the proteins derived from a defatted meal of *Hermetia illucens* larvae had no negative effect on the technological properties of chicken meat.

From the feeding point of view, a partial replacement of soybean proteins with the proteins obtained from the defatted meal of *Hermetia illucens* in the initial feed mixture (starter) up to 25% and in the rearing feed mixture up to 15% would be technologically justified.

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