Feeds of the Future and their Influence on Pork and Chicken Meat Quality

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Introduction: In most Western countries, soybean is the customary protein source in chicken and swine diets. However, the soybean market remains dominated by strong demanding (e.g. China, EU) and supplying (e.g. Argentina, USA) countries (Boerema et al., 2016); in addition, soybeans contribute to environmental degradation via their high land occupation (Mungkung et al., 2013), land use change and long transport distance (Meul et al., 2012). Overall, the integrated global market has environmental implications in exporting and importing countries (Boerema et al., 2016). In turn, research has turned its focus towards the potential of alternative protein sources that could be de-centrally produced independent of arable land.

Spirulina (Arthrospira platensis) and black soldier fly (Hermatia illucens) larvae are considered two soybean alternatives that are high in protein and can be produced in photobioreactors, as is the case with spriulina (Taelman, De Meester, Van Dijk, da Silva, & Dewulf, 2015), or on waste products, for black soldier fly (Newton, Sheppard, & Burtle, 2008). Therefore, this study examines the resulting meat quality from animals fed diets containing a (partial-) replacement of soybean meal through spirulina or partially de-fatted black soldier fly larval meal.

Materials & Methods: Ross 308 broilers and Pietrain x (Large White x German Landrace) barrows were fed amino acid supplemented diets (Table 1), where soybean meal was replaced between 50 – 100% by either spirulina (SP) or black soldier fly (HI). Animals were raised to a marketable condition in Germany (35 day old broilers; barrows to 110–120kg).Broilers were fed and housed in groups of six (6 boxes per diet) equaling 36 animals per diet; whereas barrows were raised in two experimental replications, where animals were individually housed and fed. Animal was the experimental unit for swine (n=16 per diet); however, due to limited material per animal, the 36 broilers were divided into animals for physicochemical analysis (n=28) and sensory analysis (n=8). In addition, ten animals per group were used for the broiler thigh fatty acid profiling.

Physicochemical parameters included: live weight, carcass weight, $pH_{slaughter}$ (20min post mortem for broiler and 45min for pork), pH_{24hr}, water holding capacity (drip loss and/or cooking loss), shear force, lipid oxidation and lean colour. All physicochemical parameters were recorded using established methods, such as an electronic pH meter (Knick, Germany), sous vide cooking to a stable core temperature (75°C for broiler, 65°C for pork) for cooking loss, MORS shear force (Baublits, Meullenet, Sawyer, Mehaffey, & Saha, 2006), TBARS for lipid oxidation (Bruna, Ordóñez, Fernández, Herranz, & De La Hoz, 2001), and a spectrophotometer (Konica Minolta, Japan) for lean colour. Fatty acid profiles (FAPs) for intramuscular fat (IMF) from broiler thighs and pork backfat were also investigated (Liu, Trautmann, Wigger, Zhou, & Mörlein, 2017).

Sensory analysis of sous vide cooked products was conducted with a trained panel (n=10), who had previous experience evaluating meat products. Assessors became familiar with the products through eight 2 hr individual training sessions, each for chicken and pork products, where a list of attributes to evaluate was established. Products were evaluated based on appearance, odour, flavour and taste, aftertaste, as well as texture.

Statistical analyses were carried out using one-way ANOVAs, exception is two-way ANOVAs for swine FAPs that included carcass fattiness as a covariate, for physicochemical parameters, mixed linear models for sensory data, with $p< 0.05$ considered as statistically significant. Standardized principal component analyses (PCA) of all significant parameters were used for a holistic characterization of the products.

Results & Discussion: The dietary protein source did statistically significantly impact chicken and pig meat quality traits (Table 2); however the magnitude of differences tended to remain small. As had been previously established by Toyomizu, Sato, Taroda, Kato, & Akiba, (2001) and Venkataraman, Somasekaran, & Becker (1994), spirulina (SP) as a dietary protein feed resulted in a dark red-orange colour in both the chicken breast and thigh meat (Figure 1). This is likely a due to the β-carotene in spirulina (Habib, Parvin, Huntington, & Hasan, 2008). As for eating quality, SP decreased the off-odour 'animal' and increased the chicken flavour and umami taste in cooked meat samples, which expanded the flavour profile established in a smaller pilot study (Altmann, Neumann, Velten, Liebert, & Mörlein, 2018) In pork, SP only affected eating quality by producing a stronger overall odour. Finally, although SP carcass weights did not differ from the control group, SP carcass weights were below those of the HI group in all experiments.

Black soldier fly larval meal (HI) resulted in heavier carcasses (and thigh weights in broilers), a decreased pH in broilers, yet a slightly increased pH_{45min} in the ham (M. glutaeus medius); HI pork products exuded less water during cooking. HI also improved eating quality compared to the control group by decreasing adhesiveness in chicken meat and increasing juiciness and overall odour in pork. Arguably, these results are minor concerning the quality, with perhaps the exception of carcass weight. This can be corroborated by Onsongo et al. (2018), who also found no zootechnical nor sensory differences between HI-fed broilers and their fishmeal-soybean meal control group. However, the sensory testing performed in the mentioned study was rudimentary at best as it consisted of semi-trained volunteers only evaluating 3 characteristics (aroma, taste, overall acceptability) on a 9-pt hedonic scale. We demonstrate that with a trained panel it is indeed possible to identify differences in eating quality; nonetheless, these are not likely noticeable to an untrained consumer.

The protein feeds influenced both FAPs in chicken thigh IMF and pork backfat. As expected given its relatively high saturated fatty acid (SFA) content (Barroso et al., 2014; Spranghers et al., 2017), HI increased the proportion of SFAs in chicken thigh meat. This is especially pertinent concerning the lauric acid content, which is three times higher in both broiler and pork samples. This could indicate that C12:0 would be suitable as a biological indicator for HI-fed meat products. Spirulina tends to maintain polyunsaturated fatty acid levels; although the levels exceed the control group in pork, they are only on par with the control in the broiler thigh IMF samples. The discrepancies could be due to the varying levels of fed soybean oil in the pork diets or due to the insect substrate, which is known to influence the larval meal fatty acid content (Spranghers et al., 2017).

Overall, the alternative protein sources lead to distinguishable products as is depicted in Figure 2. The SP-fed products are mostly characterized by sensory attributes and moderately increased PUFAs, whereas the HI-fed animals produced heavier carcasses and a drastically altered FAP in both broiler and pork. However, although the altered FAP is as expected in the broiler IMF, the result from the pork backfat should be taken with caution concerning the increased PUFA levels, as the HI diet also elevated levels of soybean oil compared to the other two groups in order to make up for caloric differences in the diets.

Conclusions: Alternative protein sources result in minor quality changes while incorporated into swine diets; however due to the increased proportion of protein in poultry diets, spirulina does result in a more intensive red-orange colour of meat. This could lead to consumer acceptance concerns at the point of purchase of raw products. Finally, the high content of lauric acid found in both broiler and pork products could indicate that this fatty acid is suitable to use as a biological indicator for animals fed black soldier fly larval meal.

Acknowledgments: This study is a part of the project 'Sustainability Transitions in food production: alternative protein sources from a socio-technical perspective', funded by the 'Niedersächsisches Vorab' through the Lower Saxony Ministry for Science and Culture, Germany.

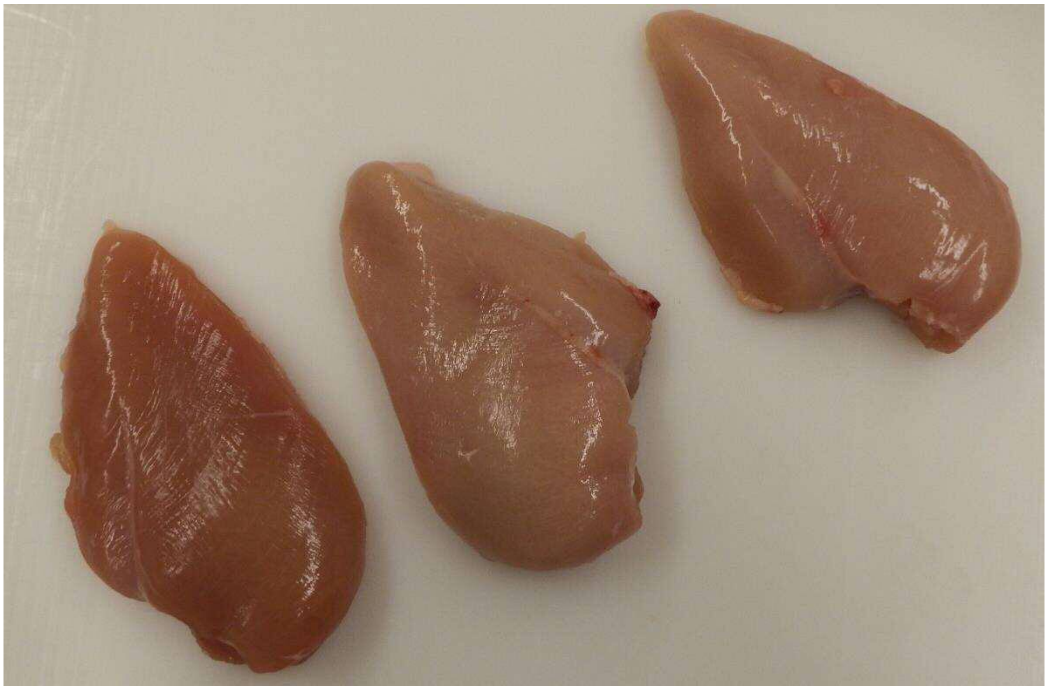

Figure 1: Photo of fresh skinless chicken breast after blooming time. From left to right: SP-fed, control, HI-fed.

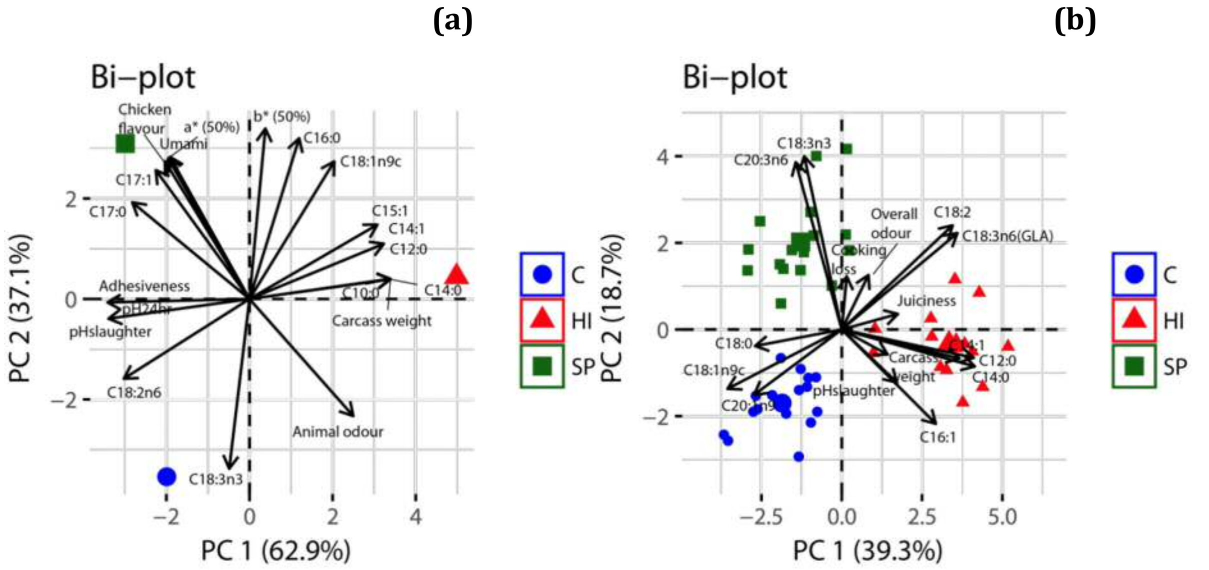

Figure 2: Bi-plots based on principal component analyses for broiler (a) and pork (b) statistically significant characteristics signified through variance analysis. Broiler analysis is based on characteristic averages per group, as animal were not used as the experimental unit.

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Table 1: Ingredient (g/kg fed) and analysed nutrient (g/kg DM) composition of control (C), *Hermetia illucens* (HI) and Spirulina (SP) diets based on physiological stage and animal type.

Animal	Broiler						Swine 25-50 kg						Swine 51-75 kg						Swine >75 kg					
Ingredients/ Diets	Starter (75%)			Grower (50%)			Replication 1 (50%)			Replication 2 (75%)			Replication 1 (50%)			Replication 2 (75%)			Replication 1 (100%)			Replication 2 (100%)		
	C	HI	SP	C	HI	SP	C	HI	SP	C	HI	S P	C	HI	SP	C	HI	SP	C	HI	SP	C	HI	SP
Wheat	390.0	390.3	392.5	360.2	396.5	398.8	365.2	369.5	371.8	365.0	371.2	376.0	394.1	397.1	399.0	394.1	398.9	402.2	416.8	427.1	431.1	416.8	426.6	430.8
Corn	163.4	195.1	196.2	180.1	198.3	199.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barley	-	-	-	-	-	-	365.2	369.5	371.8	365.0	371.2	376.0	394.1	397.1	399.0	394.1	398.9	402.2	416.8	427.1	431.1	416.8	426.6	430.8
Soybean meal	326.7	97.5	97.5	330.0	165.0	165.0	220.0	110.0	110.0	220.0	55.0	55.0	175.0	88.0	88.0	175.0	43.7	43.7	140.0	-	-	140.0	-	-
Soy oil	78.5	58.0	52.0	91.0	80.0	76.0	24.0	43.0	37.0	24.0	52.0	41.0	14.0	29.0	24.0	14.0	36.0	28.0	5.0	28.0	20.0	5.0	28.0	20.0
Premix[1] [2]	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	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
$CaCO_3$	11.0	11.0	11.0	8.0	8.0	7.0	11.0	10.0	10.0	11.0	10.0	10.0	9.0	9.0	9.0	9.0	9.0	9.0	8.0	7.0	7.0	8.0	7	7
DCP-40	11.0	8.0	11.0	10.0	8.0	9.0																		
NaCl	3.0	1.0	0.8	2.5	1.5	1.0	0.5	-	-	0.5	-	-	0.5	-	-	0.5	-	-	-	-	-	-	-	-
Hermetia	-	217.1	-	-	122.5	-	-	81.6	-	-	122.5	-	-	65.0	-	-	97.4	-	-	95.0	-	-	95.0	-
Spirulina	-	-	221.0	-	-	124.7	-	-	83.1	-	-	124.6	-	-	66.0	-	-	99.2	-	-	95.0	-	-	95.0
L-Lysine· HCl	2.5	4.2	5.8	1.8	2.8	3.6	3.4	4.8	5.4	3.3	5.1	6.0	3.0	4.1	4.6	3.0	4.4	5.1	3.1	4.8	5.5	3.1	4.8	5.5
DL-Methionine	3.6	4.2	3.5	2.5	2.9	2.5	0.1	0.7	0.4	0.7	0.8	0.4	0.4	0.2	-	-	0.3	-	-	0.3	-	-	0.2	-
L-Threonine	0.3	0.1	-	0.1	0.03	-	0.6	0.9	0.6	0.6	0.9	0.4	-	0.6	0.4	0.4	0.6	0.2	0.4	0.8	0.3	0.4	0.8	0.4
L-Arginine	-	3.5	0.2	-	1.5	-																		
L-Leucine	-	-	-	-	-	-	-	-	-	-	1.3	-	-	-	-	-	0.7	-	-	-	-	-	1.1	-
L-Valine	-	-	-	0.7	-	-																		
L-Histidine	-	-	0.6	-	-	-	-	-	-	-	-	0.8	-	-	-	-	-	0.5	-	-	-	-	-	0.6
Analysed nutrients (g/kg DM)																								
Crude protein	247.8	268.6	262.2	236.9	224.4	254.9	190.4	217.1	192.4	197.0	198.1	191.0	201.4	234.2	203.2	181.4	181.4	185.2	138.9	172.0	172.7	170.0	161.1	149.6
Ether extract	102.2	111.0	85.2	117.1	120.6	114.5	50.3	89.1	65.3	50.9	94.3	70.5	42.0	75.1	60.6	41.5	82.60	60.30	36.3	71.2	51.5	32.6	65.6	45.9
Crude fibre	-	-	-	-	-	-	56.4	55.9	40.1	56.7	54.0	38.2	45.1	38.0	52.4	62.4	52.8	48.7	43.7	49.2	39.4	53.7	46.9	42.0
Crude ash	-	-	-	-	-	-	52.3	58.5	46.1	50.8	48.3	46.4	47.9	54.2	52.0	47.7	43.9	42.5	40.2	41.3	38.5	43.8	38.5	33.0
(A)ME$_{(N)}$ (MJ/kgDM)	14.4	15.3	15.3	15.0	15.5	15.5	15.3	16.1	16.1	15.3	16.4	16.4	15.2	15.8	15.8	15.2	16.1	16.0	15.1	16.0	15.9	15.1	16.0	15.9

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[1] Added per kg of final broiler diet: 2.1 g calcium, 0.8 g sodium, 5,000 IU vitamin A, 1,000 IU vitamin D3, 30 mg vitamin E, 2.6 mg vitamin B1, 4.8 mg vitamin B2, 3.2 mg vitamin B6, 20 µg vitamin B12, 3 mg vitamin K3, 50 mg nicotinic acid, 10 mg calcium pantothenate, 0.9 mg folic acid, 100 µg biotin, 1000 mg choline chloride, 50 mg Fe as iron-II-sulfate, monohydrate, 15 mg Cu as copper-II-sulfate, pentahydrate, 120 mg Mn as manganese-II-oxide, 70 mg Zn as zinc oxide, 1.4 mg I as calcium iodate, hexahydrate, 0.28 mg Se as sodium selenite, 0.55 mg Co as alkaline cobalt-II-carbonate, monohydrate and 100 mg butylhydroxytoluol.1

[2] Supplementation of diets for growing pigs (per kg of final diet): Ca, 0.14%; P, 0.10%; Na, 0.12%; vitamin A, 4,000 IU; vitamin D3, 500 IU; vitamin E, 40 mg; thiamine, 1.5 mg; riboflavin, 6.0 mg; vitamin B6, 3 mg; vitamin B12, 30 µg; vitamin K3, 3 mg; nicotinic acid, 20.0 mg; calcium pantothenate, 12.0 mg; folic acid, 0.5 mg; biotin, 100 µg; choline chloride, 100 mg; iron, 80 mg; copper, 5 mg; manganese, 27.5 mg; zinc, 75 mg; iodine, 0.68 mg; selenium, 0.2 mg; phytase (EC 3.1.3.8), 500 FTU.

Table 2: estimated marginal means (standard error) of statistically significantly different physicochemical parameters and sensory attributes.

Characteristic	Broiler			Pork		
	C	HI	SP	C	HI	SP
Carcass weight (kg)	1.73^{b} (0.04))	1.89^{a} (0.04)	1.70^{b} (0.04)	95.08^{ab} (1.17)	97.99^{a} (1.21)	93.11^{b} (1.17)
$\mathrm{pH}_{\mathrm{slaughter}}$	6.79^{a} (0.03)	6.65^{b} (0.03)	6.71^{a} (0.03)	6.08^{b} (0.05)	6.21^{a} (0.05)	6.00^{b} (0.05)
$\mathrm{pH}_{24\mathrm{hr}}$	5.96^{a} (0.02)	5.84^{b} (0.02)	5.99^{a} (0.02)	5.41^{a} (0.03)	5.41^{a} (0.03)	5.42^{a} (0.03)
Lean colour a*	1.79^{b} (0.23)	1.95^{b} (0.23)	3.81^{a} (0.23)	2.75^{a} (0.23)	3.47^{a} (0.24)	3.01^{a} (0.23)
Lean colour b*	13.14^{b} (0.25)	14.45^{a} (0.25)	15.11^{a} (0.25)	13.83^{a} (0.30)	13.86^{a} (0.31)	13.63^{a} (0.30)
Cooking loss (%)	24.56^{a} (0.79)	27.27^{a} (0.79)	25.89^{a} (0.80)	32.4^{a} (0.30)	31.4^{b} (0.30)	32.3^{a} (0.30)
Overall odour (scale 0-100)	55.2^{a} (5.5)	57.5^{a} (5.5)	53.3^{a} (5.5)	62.3^{b} (3.2)	66.0^{a} (3.2)	66.3^{a} (3.2)
'animal' odour (scale 0-100)	14.9^{a} (2.5)	15.8^{a} (2.5)	11.1^{b} (2.5)	-	-	-
Chicken flavour (scale 0-100)	56.0^{b} (5.9)	55.7^{b} (5.9)	59.1^{a} (5.9)	-	-	-
Umami taste (scale 0-100)	18.6^{b} (3.9)	18.7^{b} (3.9)	21.8^{a} (3.9)	25.3^{a} (5.2)	27.5^{a} (5.2)	26.9^{a} (5.2)
Adhesiveness (scale 0-100)	47.8^{a} (6.7)	43.5^{b} (6.7)	48.3^{a} (6.7)	45.6^{a} (5.6)	44.1^{a} (5.6)	39.7^{a} (5.6)
Juiciness (scale 0-100)	41.2^{a} (5.8)	42.3^{a} (5.8)	41.6^{a} (5.8)	20.5^{b} (4.3)	25.6^{a} (4.3)	21.4^{b} (4.3)
Saturated fatty acids (SFA; %)	24.691^{b} (0.300)	29.420^{a} (0.300)	25.326^{b} (0.300)	39.752^{a} (0.446)	39.329^{ab} (0.418)	38.427^{b} (0.398)
C10:0 (%)	0.000^{b} (0.001)	0.049^{a} (0.001)	0.000^{b} (0.001)	0.060^{a} (0.002)	0.061^{a} (0.002)	0.055^{a} (0.002)
C12:0 (%)	0.019^{b} (0.077)	3.143^{a} (0.077)	0.034^{b} (0.077)	0.087^{b} (0.013)	0.567^{a} (0.012)	0.087^{b} (0.011)
C14:0 (%)	0.181^{b} (0.030)	1.223^{a} (0.030)	0.179^{b} (0.030)	1.156^{b} (0.041)	2.202^{a} (0.038)	1.124^{b} (0.037)
C16:0 (%)	13.670^{b} (0.293)	14.980^{a} (0.293)	15.064^{a} (0.293)	24.866^{a} (0.244)	24.262^{a} (0.226)	24.239^{a} (0.218)
C17:0 (%)	0.223^{b} (0.008)	0.154^{c} (0.008)	0.356^{a} (0.008)	0.205^{a} (0.024)	0.222^{a} (0.022)	0.216^{a} (0.022)
C18:0 (%)	9.349^{a} (0.359)	8.492^{a} (0.359)	8.468^{a} (0.359)	13.153^{a} (0.278)	11.269^{b} (0.257)	12.473^{a} (0.248)
Monounsaturated fatty acids (MUFA;%)	22.190^{b} (0.543)	24.397^{a} (0.543)	24.028^{ab} (0.543)	44.530^{a} (0.452)	39.329^{c} (0.418)	41.781^{b} (0.403)
C14:1 (%)	0.022^{c} (0.007)	0.190^{a} (0.007)	0.053^{b} (0.007)	0.009^{b} (0.003)	0.033^{a} (0.003)	0.011^{b} (0.002)
C15:1 (%)	0.013^{b} (0.007)	0.042^{a} (0.007)	0.019^{b} (0.007)	-	-	-
C16:1 (%)	0.541^{a} (0.108)	0.275^{a} (0.108)	0.492^{a} (0.108)	1.669^{b} (0.058)	1.902^{a} (0.054)	1.498^{c} (0.052)
C17:1 (%)	0.038^{b} (0.007)	0.035^{b} (0.007)	0.086^{a} (0.007)	0.182^{a} (0.018)	0.127^{a} (0.016)	0.156^{a} (0.016)
C18:1n9c (%)	18.992^{b} (0.488)	21.159^{a} (0.488)	20.641^{ab} (0.488)	38.671^{a} (0.433)	33.457^{c} (0.400)	36.378^{b} (0.386)
C20:1n9 (%)	0.112^{a} (0.011)	0.106^{a} (0.011)	0.098^{a} (0.011)	0.722^{a} (0.027)	0.529^{c} (0.025)	0.606^{b} (0.024)
Polyunsaturated fatty acids (PUFA;%)	53.120^{a} (0.588)	46.182^{c} (0.588)	50.644^{b} (0.588)	15.718^{c} (0.351)	21.873^{a} (0.324)	19.793^{b} (0.313)
C18:2 (%)	-	-	-	13.809^{c} (0.323)	19.326^{a} (0.298)	17.242^{b} (0.288)
C18:2n6 (%)	39.502^{a} (0.409)	33.741^{c} (0.409)	37.404^{b} (0.409)	-	-	-
C18:3n3 (%)	3.458^{a} (0.198)	2.946^{ab} (0.198)	2.760^{b} (0.198)	0.044^{c} (0.004)	0.052^{b} (0.003)	0.141^{a} (0.003)
C18:3n6 (%)	2.698^{a} (0.276)	3.153^{a} (0.276)	3.181^{a} (0.276)	0.980^{c} (0.026)	1.570^{a} (0.024)	1.320^{b} (0.023)
C20:3n6 (%)	0.102^{a} (0.026)	0.117^{a} (0.026)	0.125^{a} (0.026)	0.060^{b} (0.003)	0.059^{b} (0.003)	0.191^{a} (0.003)