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Effect of partial and complete substitution of lupin meal for soybean meal in diets on changes in fatty acid composition of muscle fat in broiler chickens

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Abstract: The experiment aimed to determine the effect of 50% (LS50) or 100% (LS100) substitution of lupin protein (variety Zulika) for soybean protein in feed mixtures intended for the nutrition of broiler chickens, on muscular fat quality (composition of fatty acids). There were established three groups of chickens for fattening with 80 individuals each (control group C and experimental groups). After the 34-day fattening period chicken breast and thigh muscles were analysed to find out the fatty acid composition. Lupin protein-based diets had a positive effect on the muscle quality of fattened chickens due to changes in fatty acid composition, compared to soya protein-based diets. The feeding of lupin-based diets to broiler chickens resulted in the reduction of saturated fatty acids ($P \leq 0.05$) by 14% in LS50 group and 17% in LS100 group and increase of unsaturated fatty acids ($P \leq 0.05$) by 58% in LS50 group and 90% in LS100 group in muscle fat. The results clearly confirm that lupin-based diet increases the dietary value of chicken meat as one of the most important protein sources in human nutrition.

Keywords: broilers; fattening; meat quality; UFA; SFA

Lupin seeds, by their nutrient composition, represent an important source of proteins that can be successfully used as a protein component in feed mixtures intended to feed farm animals. In poultry nutrition crude protein is the most costly item. Importance is attached not only to the amount of proteins but also to the source and composition because protein and essential amino acids are indispensable for growth (Hofmann et al. 2019). Finding protein feeds capable to completely or partially replace soya products became very important after the ban on feeding animal protein to livestock animals. The high price of imported soya, non-self-sufficiency of this commodity but also the issue of genetically modified crops (which include most varieties of soybean, de Vos and Swanenburg 2018) are increasing pressure on the farmers and feed producers for using other protein sources. From this point of view, legumes, among which cultivated species of lupins are included (the genus *Lupinus*), may be considered as potential protein crops. White lupin varieties contain in fact comparable amounts of crude protein like soybeans as documented by Martinez-Villaluenga et al. (2006). Strakova et al. (2006) reported that the protein content of lupin seeds can even be increased by dehulling, when crude protein content may be increased by about 20–30%, depending on the variety. Lupin seed oil

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is also an important source of polyunsaturated fatty acids (PUFA) (Zapletal et al. 2015), mainly the n-3 and n-6 group. For these reasons, the seeds of cultivated white lupin varieties are a subject of great interest as an alternative source of dietary protein in feed mixtures for animals, e.g., for fattening pigs (Zraly et al. 2008; Kasprowicz-Potocka et al. 2016) or for rabbits (Volek et al. 2018) and broiler chickens (Suchy et al. 2010). Lupin seeds find a wide range of utilisation in feed mixtures for poultry nutrition (Jeroch et al. 2016), primarily in the fattening of broiler chickens (Olkowski 2018), or rearing of laying hens (Rutkowski et al. 2017).

Together with the growing population the demand for foodstuffs of animal origin is identically increasing. For this reason, there is a rising demand for high meat production all over the world and broiler chickens have been selected mainly due to fast growth. Animal and plant ingredients are the main sources of protein used in poultry diets and they vary in digestibility and amino acid composition (Kim et al. 2011). Europe is not self-sufficient in soybean production, so there exist efforts to find other high-quality sources of proteins e.g. pea (Laudadio and Tufarelli 2011), peanuts (Toomer et al. 2020) and lupin (Jeroch et al. 2016). Other scientists are trying to find out if it is possible to influence the meat quality by quantitative feed restriction (Tumova et al. 2022) and thus reduce the costs and simultaneously maintain the quality of poultry products.

Consumer awareness of the quality and safety of food products is rapidly increasing. To maintain this demand, alternative solutions have to be taken to decrease the cost of feedstuffs and to increase the quality of poultry products. Moreover, the production and international trade in poultry meat have been greatly affected by the global *SARS-CoV-2 virus* (COVID-19) pandemic in the last two years, which has mainly had a negative effect on feed chain and agricultural sector (Hafez and Attia 2020). The COVID-19 pandemic has affected the whole foodstuff market and almost all commodities, not excluding poultry. Another hazard affecting the commercial activities of poultry farmers is avian influenza caused by *influenza A viruses* that primarily affects birds and can only rarely be transmitted to certain mammalian species. Particular attention is currently paid to its highly pathogenic forms such as H5N1 (Webster et al. 2007). Its outbreaks occur regularly and the destruction of poultry flocks has a very negative economic impact. This not-so-positive economic situation is pushing farmers to reduce as much as possible the costs of breeding, not least the cost of feed mixtures. And one of the solutions could be to search for alternative low-cost protein sources. The aim of this study was to verify whether the replacement of soya extracted meal with meal from dehulled lupin seeds would affect and change the fatty acid (FA) fat composition of the breast and thigh muscles of fattened chickens.

MATERIAL AND METHODS

Experimental birds, feeding and management

The Zulika variety belonging to the white lupin variety group was used for the experiment. The chickens (ROSS 308 genotype) were fattened in a hall on bedding according to the technological instructions for fattening ROSS 308 broiler chickens in a temperature regime of 31–21 °C, with controlled light and ventilation. Chickens were divided into three groups: the control group (C) and two experimental groups (LS50 and LS100). Each group included 80 individuals of one-day-old chickens (20 males and 20 females; two replications, 20 chickens per replication). Chickens were fed three complete feed mixtures *ad libitum* during the 34-day fattening period: starter BR1 (day 1–12), grower BR2 (day 13–27), and finisher BR3 (day 28–34). Diets were formulated to meet the requirements of fattened chickens. The feed mixtures used for the group C contained only soybean meal as a protein source; in the diets of experimental groups, 50% (LS50) and 100% (LS100) of the soybean meal was replaced on protein content basis with an alternative protein source – meal of whole dehulled white lupin seeds. Commercially produced complete feed mixtures were administered in a mash form from the trough feeders (with the exception of BR3 which was in the form of pellets). Water was administered via bell drinkers *ad libitum*. The nutritional composition of the feed mixtures is presented in Table 1. The specific diet formula is protected with a utility model. The ingredient composition is the know-how of the producer and the firm would not like to publish it.

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Table 1. Nutrient composition of feed mixtures

	BR1 (starter)			BR2 (grower)			BR3 (finisher)		
	C	LS50	LS100	C	LS50	LS100	C	LS50	LS100
Crude protein (g/kg)	247.1	236.7	243.2	217.1	210.6	204.1	200.1	183.4	191.5
Fat (g/kg)	54.2	55.6	65.0	65.9	73.2	78.3	62.7	79.1	90.1
Fibre (g/kg)	26.85	23.64	28.7	25.8	23.4	42.6	24.4	29.9	22.9
NFES (g/kg)	607.7	622.3	606.5	630.1	644.1	626.9	665.4	663.0	651.4
Starch (g/kg)	428.1	434.0	425.3	444.3	469.0	447.7	492.8	487.3	479.3
Organic matter (g/kg)	935.8	938.2	943.4	938.8	951.3	951.9	952.6	955.3	955.9
Ash (g/kg)	64.2	61.8	56.5	61.1	48.8	48.2	47.4	44.7	44.1
GE (MJ/kg)	19.1	18.9	19.3	19.4	19.6	19.6	19.3	19.5	19.7
ME (MJ/kg)	13.2	13.3	13.5	13.4	13.7	13.6	13.5	13.7	14.1

BR1, BR2, BR3 = types of feed mixtures; C = control group; GE = gross energy; LS50/100 = experimental groups (fed experimental diets with 50% or 100% substitution of lupin seed meal for soybean meal); ME = metabolizable energy; NFES = nitrogen free extract substances

Chemical analyses

At 34 days of age, the fattening process was completed. After chicken slaughtering, the breast and thigh meat was taken from each chicken group for further analysis (n = 20; 10 males and 10 females). For the chemical analysis of poultry meat the whole pectoral muscle and the whole thigh from the left part of the broiler chicken carcass were homogenized. The fat extraction was carried out according to the Soxhlet method. The extraction of fat from meat for fatty acid (FA) determination was carried out according to Hara and Radin (1978) – hexane-isopropanol extraction. FAs were determined by gas chromatography via the gas chromatograph Shimadzu GC-2010 (Shimadzu, Kyoto, Japan) with automatic injection system and flame ionization detector. For the determination of FAs, a VB-Wax separation capillary column was used (length 60 m, column internal diameter 0.25 mm, thickness of polyethylene glycol film 0.25 μm) with the addition of an internal standard (methyl pentadecanoate). FAs from groups of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) detected in the feed mixtures and/or chicken muscles are listed individually in Results and in Table 2.

Welfare statement

Prior to the start of the experiment, the experimental project No. 57-2015 "Optimization of protein nutrition of poultry based on white lupine (*Lupinus albus*) seed varieties" was approved in order to ensure the welfare requirements of animals.

Statistical analyses

Differences in the FA composition of three dietary groups were evaluated using two-way analysis of variance (ANOVA) followed by post-hoc multiple comparisons (Tukey HSD test), with the significance level at $P \leq 0.05$. To assure normal distribution of the data the single-factor dispersion analysis was used (ANOVA – analysis of variance). All analyses were performed using the Unistat v5.6 (Unistat Ltd, London, UK) for MS Excel.

RESULTS

SFA

For SFA (C10:0, C13:0, C23:0) there were no significant differences between the mean values in the breast and thigh muscle fat of broiler chickens from both experimental groups (LS50, LS100) compared to group C (Table 3). For most other SFA (C8:0, C12:0, C14:0, C16:0, C17:0, C18:0) lower mean values were demonstrated in the fat of both the breast and thigh muscle in experimental groups ($P \leq 0.05$) compared to C group. SFA C20:0 and C24:0 were shown ($P \leq 0.05$) to have higher average contents in the muscle fat of experimental

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Table 2. Fatty acid (FA; g/100 g of fat) composition of feed mixtures and lupin seeds

FA	BR1 (starter)			BR2 (grower)			BR3 (finisher)			Dehulled lupin seeds
	C	LS50	LS100	C	LS50	LS100	C	LS50	LS100	
C8:0 (caprylic acid)	0	0	0	0.01	0	0	0.01	0.01	0.01	0
C10:0 (capric acid)	0	0	0	0.02	0.02	0.01	0.03	0.03	0.03	0
C12:0 (lauric acid)	0.01	0.01	0.01	0.07	0.04	0.03	0.08	0.07	0.07	0.01
C13:0 (tridecylic acid)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01
C14:0 (myristic acid)	0.10	0.08	0.10	0.61	0.36	0.31	0.63	0.65	0.69	0.08
C16:0 (palmitic acid)	0.14	0.28	4.95	10.89	9.07	8.64	10.96	11.98	13.12	6.61
C17:0 (margaric acid)	0.07	0.07	0.07	0.26	0.17	0.15	0.26	0.25	0.27	0.07
C18:0 (stearic acid)	1.29	1.32	1.01	5.42	3.41	2.98	5.51	5.21	5.61	0.74
C20:0 (arachidic acid)	0.14	0.15	0.26	0.12	0.21	0.27	0.10	0.18	0.25	0.68
C23:0 (tricosanoic acid)	0.02	0.02	0.04	0.03	0.03	0.05	0.03	0.04	0.05	0.16
C24:0 (lignoceric acid)	0.09	0.12	0.28	0.07	0.18	0.28	0.07	0.18	0.26	0.53
ΣSFA	1.84	2.05	6.72	17.50	13.48	12.72	17.68	18.61	20.37	9.33
C14:1 (myristoleic acid)	0	0	0	0.07	0.04	0.03	0.08	0.08	0.08	0
C15:1 (pentadecenoic acid)	0.01	0	0	0	0	0.01	0	0.01	0.01	0.01
C16:1 (palmitoleic acid)	0.10	0.11	0.19	0.89	0.67	0.60	0.93	1.19	1.30	0.54
C17:1 (heptadecenoic acid)	0.03	0.03	0.03	0.14	0.10	0.09	0.14	0.16	0.17	0
C18:1n9 (oleic acid)	4.32	6.03	7.05	8.10	9.76	11.40	7.66	11.26	13.74	18.71
C20:1n9 (gadoleic acid)	0.13	0.33	1.22	0.32	0.93	1.33	0.34	0.93	1.35	5.19
C22:1n9 (erucic acid)	0.02	0.11	0.51	0.02	0.29	0.47	0.01	0.24	0.41	2.22
C24:1n9 (nervonic acid)	0.09	0.09	0.10	0	0	0	0	0	0	0
ΣMUFA	4.70	6.70	9.11	9.54	11.80	13.94	9.16	13.86	17.06	27.94
C18:2n6 (linoleic acid)	20.08	21.20	16.36	9.00	16.70	16.74	11.31	12.87	13.66	13.63
C18:3n6 (γ-linolenic acid)	0.01	0.02	0.09	0.02	0.01	0.01	0.01	0.02	0.02	0
C20:2n6 (eicosadienoic acid)	0.02	0.03	0.08	0.08	0.10	0.12	0.09	0.14	0.16	0.20
C20:3n6 (eicosatrienoic acid)	0.01	0.02	0.03	0.03	0.02	0.02	0.03	0.04	0.04	0
C22:2n6 (docosadienoic acid)	0.02	0.01	0.01	0.08	0.06	0.06	0.09	0.12	0.14	0.20
C22:4n6 (docosatetraenoic acid)	0.10	0.11	0.07	0	0.02	0.02	0.03	0.03	0.04	0.04
Σn-6	20.24	21.39	16.62	3.00	16.91	16.95	11.57	13.22	14.05	14.98
C18:3n3 (α-linolenic acid)	2.15	2.82	3.53	1.32	2.69	3.37	1.03	2.03	2.66	8.36
C20:3n3 (dihomo γ-linolenic acid)	0.22	0.01	0.03	0.03	0.03	0.04	0.03	0.04	0.04	0.09
C20:5n3 (eicosapentaenoic acid)	0.17	0.21	0.55	0.05	0.34	0.49	0.04	0.26	0.41	2.69
C22:6n3 (docosahexaenoic acid)	0.01	0.01	0.03	0.03	0.02	0.01	0.02	0.02	0.04	0.09
C22:5n3 (docosapentaenoic acid)	0.11	0.12	0.29	0.08	0.19	0.29	0.08	0.18	0.27	0
Σn-3	2.66	3.16	4.43	1.50	3.27	4.19	1.19	2.53	3.41	11.77

BR1, BR2, BR3 = types of feed mixtures; C = control group; LS50/100 = experimental groups (fed experimental diets with 50% or 100% substitution of lupin seed meal for soybean meal); MUFA = monosaturated fatty acids; SFA = saturated fatty acids

groups of chickens (LS50 and LS100) compared to C group and the amount of these FAs was increasing together with the increasing level of lupin protein in the diet. Contrarily, the total content of SFA was significantly lower in both the breast and thigh muscle fat ($P \leq 0.05$) (mean value 21.56 and 21.07 g/100 g of fat in LS50 and LS100, resp.) compared to C group (mean value 24.55 g/100 g of fat). The most represented SFA in the muscle fat (breast and thigh) of broiler chickens were C16:0 (mean value 16.46 and 15.83 g/100 g of fat in LS50 and LS100, resp.) and C18:0 (mean value 4.12 and

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Table 3. Mean values of saturated fatty acids (SFA; g/100 g of fat) content in chicken muscles (± standard deviation)

SFA	C		LS50		LS100	
	breast	thigh	breast	thigh	breast	thigh
C8:0 (caprylic acid)	$0.007^{a} \pm 0.005$	0.006 ± 0.005	0.005 ± 0.005	0.006 ± 0.005	$0.004^{b} \pm 0.005$	0.004 ± 0.007
C10:0 (capric a.)	0.012 ± 0.004	0.010 ± 0.000	0.010 ± 0.000	0.011 ± 0.003	0.010 ± 0.000	0.011 ± 0.003
C12:0 (lauric a.)	$0.052^{a} \pm 0.004$	$0.049^{a} \pm 0.006$	$0.038^{b} \pm 0.006$	0.043 ± 0.016	$0.038^{b} \pm 0.007$	$0.035^{b} \pm 0.005$
C13:0 (tridecylic a.)	0.010 ± 0.000	0.010 ± 0.000	0.010 ± 0.000	0.010 ± 0.000	0.010 ± 0.000	0.011 ± 0.003
C14:0 (myristic a.)	$0.683^{a} \pm 0.067$	$0.617^{a} \pm 0.051$	$0.517^{b} \pm 0.078$	$0.521^{bc} \pm 0.038$	$0.491^{b} \pm 0.045$	$0.474^{bd} \pm 0.024$
C16:0 (palmitic a.)	$18.892^{a} \pm 0.960$	$18.218^{a} \pm 1.874$	$16.219^{b} \pm 2.086$	$16.696^{b} \pm 1.343$	$16.001^{b} \pm 1.946$	$15.653^{b} \pm 0.829$
C17:0 (margaric a.)	$0.254^{a} \pm 0.029$	$0.226^{a} \pm 0.019$	$0.200^{b} \pm 0.018$	$0.206^{b} \pm 0.026$	$0.216^{b} \pm 0.031$	$0.213^{b} \pm 0.018$
C18:0 (stearic a.)	$5.174^{a} \pm 0.675$	$4.577^{a} \pm 0.721$	$4.181^{b} \pm 0.411$	$4.056^{b} \pm 0.319$	$4.452^{b} \pm 0.638$	$4.039^{b} \pm 0.255$
C20:0 (arachidic a.)	$0.047^{bd} \pm 0.007$	$0.041^{bd} \pm 0.014$	$0.056^{bc} \pm 0.007$	$0.056^{bc} \pm 0.006$	$0.070^{a} \pm 0.008$	$0.082^{a} \pm 0.012$
C23:0 (tricosanoic a.)	0.013 ± 0.005	0.010 ± 0.000	0.014 ± 0.009	0.012 ± 0.004	0.012 ± 0.008	0.008 ± 0.009
C24:0 (lignoceric a.)	$0.106^{b} \pm 0.014$	$0.072^{bd} \pm 0.010$	$0.142^{b} \pm 0.043$	$0.095^{bc} \pm 0.019$	$0.193^{a} \pm 0.077$	$0.113^{a} \pm 0.013$
ΣSFA	$25.251^{a} \pm 1.624$	$23.835^{a} \pm 2.608$	$21.408^{b} \pm 2.469$	$21.711^{b} \pm 1.583$	$21.495^{b} \pm 2.622$	$20.641^{b} \pm 0.814$

C = control group; LS50/100 = experimental groups (fed experimental diets with 50% or 100% substitution of lupin seed meal for soybean meal)

[a–d]Means in the same row differ significantly, $P \leq 0.05$; $n = 20$

4.25 g/100 g of fat in LS50 and LS100, resp.), which were dominant compared to other SFA and their values were the highest in C group (mean value 18.56 g/100 g of fat for C16:0 and 4.87 g/100 g of fat for C18:0).

MUFA

The MUFA values in the muscles of broiler chickens are shown in Table 4. The analysis showed no evidence of nervonic acid (C24:1n9) in muscle fat. In C15:1, there was no evidence of a difference in the pectoral muscle fat of C, LS50 and LS100 groups. In general, the fat of the chicken muscles contained significantly ($P \leq 0.05$) more MUFA in total (mean value 37.28 and 38.22 g/100 g of fat in LS50 and LS100, resp.) compared to control group (mean value 21.49 g/100 g of fat). This positive effect was also proved with incresing values of C18:1n9, C20:1n9 and C22:1n9. Conversely, for MUFA C14:1, C16:1 and C17:1 their values were higher in the muscle fat of chickens in C group. The most represented MUFA were C16:1n9 and C18:1n9 in both

Table 4. Mean values of monounsaturated fatty acids (MUFA; g/100 g of fat) content in chicken muscles (± standard deviation)

MUFA	C		LS50		LS100	
	breast	thigh	breast	thigh	breast	thigh
C14:1 (myristoleic acid)	$0.177^{a} \pm 0.010$	$0.167^{a} \pm 0.014$	$0.137^{bc} \pm 0.030$	$0.138^{bc} \pm 0.017$	$0.116^{bd} \pm 0.020$	$0.122^{bd} \pm 0.019$
C15:1 (pentadecenoic a.)	0.010 ± 0.000	$0.010^{b} \pm 0.000$	0.013 ± 0.007	$0.014^{a} \pm 0.008$	0.011 ± 0.003	$0.009^{b} \pm 0.003$
C16:1 (palmitoleic a.)	$4.390^{a} \pm 0.353$	$4.197^{a} \pm 0.271$	$3.703^{bc} \pm 0.737$	$4.109^{a} \pm 0.496$	$3.212^{bd} \pm 0.532$	$3.490^{b} \pm 0.469$
C17:1 (heptadecenoic a.)	$0.190^{a} \pm 0.023$	$0.172^{a} \pm 0.015$	$0.160^{b} \pm 0.019$	$0.148^{b} \pm 0.047$	$0.161^{b} \pm 0.018$	$0.154^{b} \pm 0.021$
C18:1n9 (oleic a.)	$17.058^{b} \pm 0.739$	$15.885^{bd} \pm 0.875$	$31.557^{a} \pm 3.468$	$33.309^{bc} \pm 1.741$	$32.268^{a} \pm 4.433$	$35.111^{a} \pm 1.455$
C20:1n9 (gadoleic a.)	$0.361^{bd} \pm 0.030$	$0.335^{bd} \pm 0.048$	$0.568^{bc} \pm 0.067$	$0.567^{bc} \pm 0.054$	$0.791^{a} \pm 0.099$	$0.805^{a} \pm 0.037$
C22:1n9 (erucic a.)	$0.015^{bd} \pm 0.005$	$0.018^{bd} \pm 0.006$	$0.056^{bc} \pm 0.007$	$0.063^{bc} \pm 0.010$	$0.097^{a} \pm 0.013$	$0.084^{a} \pm 0.030$
ΣMUFA	$22.200^{b} \pm 0.927$	$20.782\,5^{bd} \pm 0.960$	$36.197^{a} \pm 4.231$	$38.346^{bc} \pm 2.108$	$36.655^{a} \pm 4.601$	$39.773^{a} \pm 1.708$

C = control group; LS50/100 = experimental groups (fed experimental diets with 50% or 100% substitution of lupin seed meal for soybean meal)

[a–d]Means in the same row differ significantly, $P \leq 0.05$; $n = 20$

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Table 5. Mean values of n-6 fatty acid (FA; g/100 g of fat) content in chicken muscles (± standard deviation)

FA	C		LS50		LS100	
	breast	thigh	breast	thigh	breast	thigh
C18:2n6 (linoleic acid)	11.860 ± 0.629	11.863^{b} ± 0.795	11.573 ± 1.198	12.520^{a} ± 0.984	11.134 ± 1.312	11.604^{b} ± 0.430
C18:3n6 (γ-linolenic a.)	0.125^{a} ± 0.018	0.118 ± 0.020	0.099^{b} ± 0.017	0.111 ± 0.016	0.102^{b} ± 0.016	0.109 ± 0.019
C20:2n6 (eicosadienoic a.)	0.136^{b} ± 0.014	0.113^{b} ± 0.009	0.151 ± 0.026	0.127^{a} ± 0.016	0.155^{a} ± 0.024	0.127^{a} ± 0.009
C20:3n6 (eicosatrienoic a.)	0.139 ± 0.010	0.097 ± 0.014	0.121 ± 0.049	0.108 ± 0.020	0.138 ± 0.022	0.094 ± 0.010
C22:2n6 (docosadienoic a.)	0.008 ± 0.004	0.006^{b} ± 0.005	0.009 ± 0.005	0.013a ± 0.006	0.010 ± 0.006	0.012^{a} ± 0.011
C22:4n6 (docosatetraenoic a.)	0.115 ± 0.015	0.076 ± 0.009	0.121 ± 0.044	0.080 ± 0.016	0.122 ± 0.038	0.077 ± 0.012
Σn-6	12.862^{a} ± 0.580	12.616 ± 0.792	12.459 ± 1.088	13.170^{a} ± 1.155	11.703^{b} ± 1.389	12.087^{b} ± 0.377

C = control group; LS50/100 = experimental groups (fed experimental diets with 50% or 100% substitution of lupin seed meal for soybean meal)

$^{a–d}$Means in the same row differ significantly, $P \leq 0.05$; $n = 20$

the pectoral and thigh muscle fat but only at C18:1n9 the values were significantly higher in experimental groups (mean value 32.44 and 33.69 g/100 g of fat in LS50 and LS100, resp.) compared to C group (mean value 16.48 g/100 g of fat).

PUFA n-6

The results of the fat analysis for n-6 FAs show (Table 5) that the substitution of lupin meal for soybean meal in diets had the lowest effect on their content in chicken muscle fat. A decrease was observed in the total amout of n-6 FAs in chicken muscle fat mainly in LS100 group (11.90 g/100 g of fat) compared to the control group (12.84 g/100 g of fat). For individual n-6 FAs, this decreasing tendency was also demonstrated for C18:2n6 and C18:3n6. The opposite trend, i.e. higher values of n-6 FAs in muscle fat in experimental chickens, was observed in C20:2n6 and C22:2n6. The results indicated that the administration of lupin diets did not affect the mean C20:3n6 and C22:4n6 content and that the most represented from this group was C18:2n6 FA, both in the breast and thigh muscle fat.

PUFA n-3

The results in Table 6 show that diets containing lupin meal significantly increased the n-3 FA content in muscle fat in experimental groups (LS50 and LS100), both in the breast and thigh muscle. The experiment showed that together with the increasing content of lupin meal in diet the total amount of n-3 FAs is similarly increasing ($P \leq 0.05$) in both the breast and thigh fat of fattened chickens (mean values 1.94 and 2.31 g/100 g of fat in LS50 and LS100, resp.) compared to 1.24 g/100 g of fat (C). In breast and thigh fat, C18:3n3 was

Table 6. Mean values of n-3 fatty acid (FA g/100 g of fat) content in chicken muscles (± standard deviation)

FA	C		LS50		LS100	
	breast	thigh	breast	thigh	breast	thigh
C18:3n3 (α-linolenic acid)	1.047^{bd} ± 0.082	1.058^{bd} ± 0.076	1.587^{bc} ± 0.227	1.740^{bc} ± 0.155	1.932^{a} ± 0.203	2.083^{a} ± 0.078
C20:3n3 (dihomo γ-linolenic a.)	0.020^{a} ± 0.000	0.020^{a} ± 0.000	0.020^{a} ± 0.021	0.009^{b} ± 0.014	0.000^{b} ± 0.000	0.000^{b} ± 0.000
C20:5n3 (eicosapentaenoic a.)	0.038^{b} ± 0.007	0.033^{bd} ± 0.007	0.054 ± 0.007	0.049^{bc} ± 0.007	0.071^{a} ± 0.010	0.065^{a} ± 0.011
C22:6n3 (docosahexaenoic a.)	0.062^{b} ± 0.012	0.035^{b} ± 0.007	0.079^{b} ± 0.036	0.048^{b} ± 0.019	0.105^{a} ± 0.043	0.064^{a} ± 0.028
C22:5n3 (docosapentaenoic a.)	0.110^{b} ± 0.014	0.069 ± 0.007	0.141^{b} ± 0.043	0.141 ± 0.180	0.187^{a} ± 0.067	0.117 ± 0.012
Σn-3	1.276^{bd} ± 0.073	1.215^{bd} ± 0.075	1.879^{bc} ± 0.192	1.986^{bc} ± 0.260	2.294^{a} ± 0.285	2.328^{a} ± 0.073

C = control group; LS50/100 = experimental groups (fed experimental diets with 50% or 100% substitution of lupin seed meal for soybean meal)

$^{a–d}$Means in the same row differ significantly, $P \leq 0.05$; $n = 20$

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the most represented n-3 FA (mean values 1.67 and 2.01 g/100 g of fat in LS50 and LS100, resp.) compared to 1.06 g/100 g of fat (C), while other n-3 FA (C20:3n3, C20:5n3, C22:6n3 and C22:5n3) were at concentrations below 1%. The dependence on lupin content was not proved only in C20:3n3, which showed the decreasing trend in experimental groups (LS50 and LS100).

Feeding lupine-based diets had a positive effect on the ratio of n-3 : n-6 PUFA in muscle fat, which was 1 : 10.1 for breast and 1 : 10.4 for thigh in C group, 1 : 6.6 for both breast and thigh in LS50 group and 1 : 5.1 for both breast and thigh in LS100 group. Where the basic rule is valid – the narrower the ratio of n-3 : n-6, the better the fat quality from the dietary point of view.

DISCUSSION

Human demand for animal origin foodstuffs is growing as the world population increases every day. Hence, fast-growing chickens could be the answer to cover this demand. The results of our study indicated a significant effect of lupin meal diets on the composition of FAs of muscle fat in the breast and thigh muscle of fattened chickens. In experimental groups, feeding lupin diets resulted in a significant reduction in both the breast and thigh muscle fat SFA compared to the control group. The present study is in agreement with Alloui et al. (1994) being concerned with the nutritive value of three varieties of lupin seeds as well as Roth-Maier and Paulicks (2003), who made a trial with a total of 108 broiler chickens fed seeds of sweet blue lupins. They found out that up to 20% of lupin seeds can be included in broiler diets in replacement of soybean meal without impairing growth performance and feed-to-gain efficiency and composition of meat. Further, in experimental groups of the present study, feeding dehulled lupin diets had a positive effect on the ratio of n-3 : n-6 PUFA and it also increased the content of MUFA and n-3 PUFA. and therefore it can be considered a valuable alternative source of proteins and fat, which is proved to be able to support productions, while improving the quality of animal products as mentioned also by Struti et al. (2020).

Lupin exists in many varieties which can differ in the content of amino acids, fatty acids and amount of antinutritive substances; e.g. Lee et al. (2016) used blue lupin (*Lupinus angustifolius*) as the main protein source in the diets for laying hens and they found its beneficial effect on the weight gain, size and number of eggs (yolk colour included). They did not find any significant differences between the groups fed soya or lupin protein. But contrarily to the present study, they did not focus on the quality of muscles and fatty acid composition. The results of the present study showed that lupin meal diets had influenced the composition of FAs in the breast and thigh muscle of fattened chickens. In experimental groups of chickens, feeding lupin diets resulted in a significant reduction of SFA in both the breast and thigh muscle fat compared to control chickens, particularly for (C16:0) by 13% in LS50 group and 17% in LS100 group and (C18:0) by 18% (LS50) and 15% (LS100). The total SFA content in the muscle fat of the experimental chickens decreased by 14 and 17% in experimental LS50 and LS100 groups, resp.

In the study by de Oliveira et al. (2021), the authors tried to influence the performance and meat and egg quality of broiler chickens and laying hens by using olive pomace. The olive pomace contains many important substances from the nutritional point of view, it can be a significant source of fatty acids (12–22%), mostly the MUFA. Even the inclusion of up to 10% of olive pomace in the diet of broiler chickens does not negatively affect the performance and improves the quality of meat, such as odour and flavour, and increases MUFA and reduces SFA. Similarly, when lupin diets were administered to fattened chickens in the present study, the MUFA content of muscle fat increased significantly in both breast and thigh muscles, mainly the content of C18:1n9 by 97% in experimental LS50 group and by 104% in LS100 group. From these results it is apparent that even 50% substitution of lupin for soybean is sufficient to increase the level of this, for humans very important and healthy, fatty acid. Total MUFA content in the muscle fat of experimental chickens increased by 73–78% depending on the portion of substitution: a higher level of lupin means a higher content of MUFA in meat.

Also Buccioni et al. (2020) tried to find some alternative to soybean protein for feeding poultry. In their study they used residual meal from cardoon seed oil which is suitable for animal feeding due to its protein content. Feeding experimentally forty-five Kabir chickens was performed. Chickens

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were divided into three groups and fed three diets containing soybean meal (control) and cardoon meal which partially (16%) or completely replaced the soybean protein. Production performances, animal welfare, dressing out and meat colour were evaluated and no statistical differences were found between groups. In the present study, no definite changes in muscle fat content were observed for n-6 FA in experimental chickens. Obtained results did not conclusively confirm the effect of lupin diets on the n-6 FA content of chicken muscle fat. The results of the present study are in accordance with the findings of Olkowski (2018). In his study, chickens fed a lupin-based diet did not show any changes in carcass quality or yield of breast muscle and leg quarters, but they showed an increased ($P \leq 0.05$) carcass proportion of giblets and fat pad. Also, lupin meal slightly increased the muscle fat content ($P \leq 0.05$), but it is noteworthy that the fat from lupin meal fed broilers had a more convenient profile of fatty acids. Olkowski (2018) also recommended not to feed the yellow lupin meal-based diet for broilers during the first three weeks of growth because lupin is not so nutritional as soybean. Results from the group fed the feed mixture based exclusively on lupin protein (LS100) from the present study do not confirm this theory because there were not found any differences or deceleration in chicken growth.

Very important for human nutrition are n-3 FAs and their proportion in diet. The present study proves that the administration of lupin diets had a positive effect on the n-3 : n-6 ratio in muscle fat which decreased from 1 : 10 in the control group to 1 : 5 in the group fed 100% substitution of soybean for lupin. A significant increase in n-3 FAs in the muscle fat of chickens fed lupin meal-based diets can be considered as highly positive. Present results showed that with increasing levels of lupin meal in the diet of experimental chickens, the levels of n-3 FA also increased. Improvement of n-3 FA content was also reported by Swiatkiewicz et al. (2015), who added microalgae as one of the components to feed mixture for broilers and laying hens. Microalgae are a rich source of n-3 long-chained FAs and with their use as an additive the beneficial effect was achieved, i.e. higher levels of mainly eicosapentaenoic and docosahexaenoic acid. In the present study, the most represented n-3 FA in chicken muscle fat was α-linolenic acid. Changes in muscle fat quality were due to the unique composition of lupin fat, which is characterised by low SFA and high levels of MUFA and n-3 PUFA group. By feeding a diet containing lupin meal as the main protein source the level of total n-3 PUFA increased by 86%, so almost once more than in the group fed a soybean-based diet.

Enrichment of products with healthy fatty acids is required by consumers, whereas dietary fatty acid modification in the present study has proved to be a good way how to increase the nutritional value of poultry meat for customers. On top of that not only FA composition in muscle fat but also egg yolk fat content (Timova et al. 2020; Strakova et al. 2021) and egg yield and colour of the yolk (Krawczyk et al. 2015) can be improved by feeding lupin-based diet.

CONCLUSION

The lupin seed meal is a promising protein component of feed mixtures for fattening broiler chickens. This feed component does not negatively affect the production efficacy of feed mixture, improves the health of fattened chickens and thus increases the fattening performance. The unique composition of lupin oil leads to an increase in the quality of chicken muscle, assessed on the basis of their muscle fat composition. Qualitative changes in muscle fat (both breast and thigh) resulted in a statistically significant reduction of the total amount of SFA when lupin meal was fed in the diet and by contrast feed mixtures containing lupin meal had an effect on an increase of UFA. The increase of UFA in muscle fat was shown ($P \leq 0.05$) mainly in total MUFA and PUFA, with the highest levels of α-linolenic acid, which has a positive effect on human health. From a dietary point of view, the narrowing of the n-3 : n-6 ratio in the muscle fat of chickens fed diets containing lupin meal is considered to be positive.

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Conflict of interest

The authors declare no conflict of interest.

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