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THE PROFILE OF FATTY ACIDS IN CHICKEN'S MEAT AFTER HUMIC ACID AND PHYTOBIOTICS APPLICATION

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ABSTRACT

The aim of the present study was analysed the effect of supplying humic acids separately and humic acids in combination with phytobiotic as garlic and oregano powder on fatty acid (FA) profile of the most valuble parts of Ross 308 chicken carcass. A total of 200 pcs Ross 308 broiler chickens of mixed sex were randomly divided into 4 groups (n=50): control group (C) without supplementation, experiment group E1 (2% humic acids), E2 (80% humic acids and 20% garlic powder) and E3 (90% humic acids and 10% oregano powder). Fattening period lasted for 42 days and all groups were kept under the same conditions. After slaughter, the FA profiles of breast and thigh samples were determined. In comparison with control group, FA composition of breast and thigh muscle, 7 out of 15 fatty acids was affected ($P \leq 0.05$) by dietary supplementation with humic acid (E1), combination of humic acid with garlic powder (E2) and combination of humic acid with oregano powder (E3). The most represented fatty acids in breast and thigh muscle in all experimental groups were oleic acid, palmitic acid and stearic acid. Comparing breast with thigh muscle, one unanticipated finding was that breast contained slightly higher amounts of total SFA (36.41 to 38.47% in breast vs. 32.63 to 34.20% in thigh). Besides, breast muscle was found to contain slightly lower proportion of total MUFA (49.01 to 50.27% in breast vs. 51.29 to 55.50% in thigh). Breast muscle had higher percentage of total PUFA (11.45 to 11.86% in breast vs. 7.49 to 9.87% in thigh). The results of the experiment confirmed that the addition of garlic and oregano powder in combination with humic acids can affect the fatty acid profile of chicken meat.

Keywords: broiler chicken, fatty acid, humic acids, garlic, oregano

INTRODUCTION

There has been an increased interest in recent years in ways to manipulate the fatty acid composition of meat. This is because meat is seen to be a major source of fat in the diet and especially of saturated fatty acids, which have been implicated in diseases associated with modern life **(Wood *et al*., 2003)**. Among the nutritional aspects of food, lipid content and fatty acid profile are the most important factors **(Bostami *et al*., 2017)**.
Fat and fatty acids in muscle and adipose tissues are among the major factors that influence meat quality, particularly nutritional value and palatability **(Coetzee and Hoffman, 2002)**. It has been shown that consumers prefer poultry meat and its products for several reasons. Poultry meat is considered healthier owing to its relatively lower fat content compared with other animal meat as beef or pork meats **(Leeson, 1999; Bonoli et al., 2007; Brenes and Roura, 2010)**. Unlike other animal fats, around two third of poultry fat is composed of unsaturated fatty acids, and they are belonged to omega-3 (n-3) and omega-6 (n-6) fatty acids **(Shin *et al*., 2011)**.
Poultry meat contains significant amounts of monounsaturated fatty acids (MUFA), and only a third of total fat is made up of SFA. Poultry meat also provides a valuable dietary source of long chain n-3 PUFA, including α-linolenic acid (ALA, 18:3 n-3), eicosapentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3). In most Western countries, where fish consumption is relatively low, poultry meat may thus represent an important source of n-3 FAs **(Marangoni *et al.*, 2015)**. Compared with other types of meat, n-6 FAs, especially linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6), can be found mostly in the skin **(Marangoni *et al.*, 2015)**.
Maroufyan *et al*. (2012) found out 3 n-6/n-3 PUFA ratios in chicken meat from 5.5 to 1.5. **Meluzzi *et al*. (2009)** found out the content of intramuscular fat in the breast from 1.06–1.08% and in the thigh from 2.99 to 3.48% and content of PUFA from 35.3 to 37.5% in the breast muscle and from 32.2 to 35.1% in the thigh muscle. Chicken meat had a proportion of saturated 36.4% and polyunsaturated fatty. Chicken meat had a proportion of saturated 36.4% and polyunsaturated fatty acids 21.3%. Long chain omega-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic and docosahexaenoic were observed only in dark chicken meat 23 mg.100 g^{-1} **(Carnevale de Almeida *et al*., 2006)**. In addition, the composition of poultry fat is favourable from a nutritional point of view, compared with the low levels of PUFA and high levels of SFA in red meats **(Morales-Barrera *et al*., 2013)**. The FA profile of poultry meat, however, depends on internal (age, gender, and genotype) and external (temperature, feeding) factors **(Starčević *et al*., 2014)**.
Performances of broilers during fattening, slaughter characteristics and meat quality are linked to pre-mortal and post-mortal factors. It is considered that diet, as the pre-mortal factor, dominantly impacts the quality of carcasses and meat with more than 30% **(Ristić *et al*., 2005; Džinić *et al*., 2011)**. The fatty acid content of broiler meat depends on the type of diet intake by the birds **(Crespo and Esteve-Garcia, 2002)**.
During the decades, antibiotics have widely been used in poultry production as a growth promoter to enhance the performance. However, in, 2006, EU and many countries have banned using antibiotics as growth promoter in animal nutrition from reason increase antibiotic resistant bacteria, the accumulation of antibiotic residues in animal products and the potential to transfer resistant strains from animals to humans via the food chain **(Stanaćev *et al*. 2011a)**. This action

encourages many investigators to search for alternatives to enhance performance **(El-Husseiny *et al*., 2008)**.
Among possible alternatives are phytogenic feed additives (PFA) which may positively affect poultry health and productivity **(Puvača *et al*., 2015)**. Many non-therapeutic substitutes (prebiotics (**Sarangi *et al*., 2016**), probiotics (**Popova, 2017**), enzymes (**Bedford and Morgan, 1996**), bee products as bee pollen (**Haščík *et al*., 2017**) and propolis (**Saeed *et al*., 2017**), humic acids (**Rath *et al*., 2006**)), especially plants extracts from a wide variety of herbs, spices and derivatives, have already been used as a feed additive in poultry. These extracts when supplemented to animals diets can play a role in supporting both performance and health status of the animal **(Manzanilla *et al*., 2001; Kostadinovic, 2013)**.
Herbs and plant extracts used in animal feed is called phytogenics feed additives (also called phytobiotics or botanicals), are defined as compounds of plant origin incorporated into animal feed to enhance livestock productivity through the improvement of digestibility and nutrient absorption, activation of feed intake and secretion of digestive secretions, immune stimulation, antibacterial, coccidiostatic, anthelmintic, antiviral or antiinflammatory activity and inhibition or particularly antioxidant properties **(Kamel, 2001; Wenk, 2003; Balunas and Kinghorn, 2005; Athanasiadou *et al*., 2007)**.
Recent studies on these compounds have shown some positive effects as antimicrobial **(Sari *et al*., 2006; Wong *et al*., 2008)**, but also in other respects such as antioxidant ability and growth promoter function (**Wei and Shibamoto, 2007; Krishan and Narang 2014; Zeng *et al*. 2015)** and regulator of the gut flora **(Jang *et al*., 2007)** in poultry production. This indicates that plant extracts can be considered as feed additives in poultry production **(Muthusamy and Sankar, 2015)**. Many phytogenic sources to replace antibiotics show promising results not only as an antimicrobial agent.
Phytogenic feed additives are incorporated into diets to improve production performance, and the quality of food derived from those animals **(Windisch *et al*., 2007)**. The large variety of plant compounds used as PFA are assembled according to their origin and treatment, such as herbs and spices (eg: garlic, anise, cinnamon, coriander, oregano, chili, pepper, rosemary and thyme) but also essential oils or oleoresins **(Kamel, 2000)**. The content of active substances in these products can vary greatly depending on what part of the plant is used (grains, leaves, roots, bark, flowers, or buds), the harvest season and geographical origin (**Windish *et al*., 2008**).
The aim of the present study was analysed the effect of supplying humic acids separately and humic acids in combination with phytobiotic as garlic and oregano powder on fatty acid (FA) profile of the most valuble parts of Ross 308 chicken carcass.

MATERIAL AND METHODS

Animals and experimental design

The experiment was realized in the experimental poultry station of Slovak University of Agriculture (SUA) in Nitra. Chickens were randomized into four groups, each containing 50 birds. In control group we used complete feed mixture without any additives. Group of chickens E1 was fed a diet containing 2 kg of preparation Humac Natur per 100 kg feed mixture. The group marked as E2 was fed a diet containing 1.6 kg of preparation Humac Natur per 100 kg feed mixture and 0.4 kg of garlic powder per 100 kg feed mixture and group E3 containing combination 1.8 kg of preparation Humac Natur per 100 kg feed mixture and 0.2 kg of oregano leaf powder per 100 kg feed mixture. The experiment was realized by methology **Haščík *et al.* (2018)**. Chickens in individual groups were stabled on deep budding, with a maximum occupation of the breeding areas 33 $kg.m^{-2}$. During the fattening period, the light regimen based on 24 h of dark was used. The temperature at the beginning of the experiment was 31-33 °C and decreased to 20-22 °C during the experiment. The temperature was maintained using electronic hen-like devices providing radiant heat.
The fattening lasted 42 days. The feeding program included three phases: starter (1^{st} – 21^{st} days of age), grower (22^{nd} – 35^{th} days of age), and finisher (36^{th} – 42^{nd} days of age). Feed and water were supplied *ad libitum.* The feed mixtures both starter and grower were produced without any antibiotics and coccidiostats. Composition of complete feed mixtures is presented in Table 1.
Humac Natur purchased from Humac s.r.o., Kosice is preparation of humic substances on base of oxihumolit contain min. 62% humic acids in dry matter, of this 48% free munic acids in dry matter, minerals and trace elements, carboxymethylcellulose complex with humic substances. Moisture was maximum 11%.
The garlic was added to the feed in the form of finely ground *Allium sativum* L. bulbs and the oregano was added as dried and finely ground of *Oreganum sativum* leaves (Vetservis a.s.).

Slaughter and measurements

At the end of the 42-d feeding period, broilers were weighed and slaughtered at the slaughterhouse of Slovak University of Agriculture in Nitra. After evisceration, the carcasses were kept at approximately 18 °C for 1 h *post mortem* and thereafter carcasses were weighed and stored at 4 °C until 24 h *post mortem*. The breast and thigh muscles were separated from each half-carcass for the determination the FA composition. The FA compositions of breast and thigh meats were determined by a direct method for fatty acid methyl ester (FAME) synthesis. The FA composition of the FAME was determined using a Gas Chromatograph (Agilent, 7890A series, USA) equipped with a flame ionization detector and a chiral capillary column (J&W Scientific, USA).

Table 1 Composition of feed mixtures

Ingredients (%)	Starter (HYD-01) (1^{st} – 21^{st} day of age)	Grower (HYD-02) (22^{nd} – 35^{th} day of age)	Finisher (HYD-03) (36^{th} – 42^{nd} day of age)
Wheat	34.00	34.00	35.82
Maize	36.00	41.00	38.00
Soybean meal (48% N)	21.30	18.70	20.00
Fish meal (71% N)	3.80	2.00	-
Dried blood	1.25	1.25	-
Ground limestone	1.00	1.05	1.10
Monocalcium phosphate	1.00	0.70	1.00
Fodder salt	0.10	0.15	0.20
Sodium bicarbonate	0.15	0.20	0.25
Lysine	0.05	0.07	0.29
Methionine	0.15	0.22	0.29
Palm kernel oil Bergafat	0.70	0.16	2.50
Premix Euromix BR 0.5%*	0.50	0.50	0.50
Nutrient composition [$g.kg^{-1}$]			
Linoleic acid	13.54	14.23	14.94
Fibre	30.16	29.91	30.52
Crude protein	210.71	190.39	170.51
MEN ($MJ.kg^{-1}$)	12.02	12.05	12.43
Ash	24.23	19.91	38.46
Ca	8.14	7.26	7.36
P	6.76	5.72	6.02
Na	1.69	1.76	1.78

*active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 20 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 20 000 mg; folic acid 400 mg; biotin 40 mg; kobalamin 8.0 mg; choline 100 000 mg; betaine 50 000 mg; Mn 20 000 mg; Zn 16 000 mg; Fe 14 000 mg; Cu 2 400 mg; Co 80 mg; I 200 mg; Se 50 mg.

Statistical analysis

A statistical analysis was computed using the ANOVA procedures of SAS software with using of Enterprise Guide 4.2 application (version 9.3, SAS Institute Inc., USA, 2008). Data were reported as mean ± standard deviation. Statistical significance was calculated using t-test. Differences between the groups were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The results of experiment with Ross 308 broiler chickens after addition of humic acid and humic acids in combination with garlic and oregano powder, which was aimed at analysed and evaluated fatty acids profile, are presented in Table 2 and 3.

Table 2 The fatty acids profile (g.100 g^{-1}) of chicken breast muscle (mean±*SD*)

Fatty acid\Group	C	E1	E2	E3
Lauric (C12:0)	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01
Myristic (C14:0)	1.23±0.04^{b}	1.27±0.03^{ab}	1.28±0.04^{a}	1.26±0.01^{ab}
Palmic (C16:0)	24.46±0.07^{b}	24.23±0.21^{c}	24.57±0.07^{a}	24.30±0.19^{bc}
Heptadecanoic (C17:0)	0.36±0.03	0.34±0.04	0.36±0.02	0.34±0.03
Stearic (C18:0)	11.16±0.20	10.94±0.34	11.02±0.24	11.12±0.16
Oleic (C18:1 cis)	40.13±1.46^{b}	40.90±1.76^{ab}	42.68±1.66^{a}	39.14±2.59^{b}
Vaccenic (C18:1 trans-11)	4.46±0.15	4.62±0.15	4.52±0.15	4.52±0.09
Linoleic (C18:2 cis)	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
Conjugated Linoleic (C18:2 n-6)	0.14±0.02	0.14±0.02	0.13±0.01	0.13±0.01
α-Linolenic (C18:3 n-3)	1.94±0.22^{ab}	1.81±0.20^{b}	1.97±0.16^{ab}	2.02±0.14^{a}
Eicosenoic (C20:1 n-9)	0.53±0.01^{a}	0.55±0.07^{a}	0.58±0.05^{a}	0.45±0.07^{b}
Arachidonic (C20:4 n-6)	1.79±0.19^{a}	1.75±0.22^{ab}	1.67±0.15^{ab}	1.42±0.33^{b}
Eicosapentaenoic (C20:5 n-3)	0.11±0.02	0.12±0.02	0.10±0.01	0.10±0.01
Docosapentaenoic (C22:5 n-3)	0.16±0.01^{a}	0.14±0.01^{b}	0.14±0.01^{b}	0.16±0.01^{a}
Docosahexaenoic (C22:6 n-3)	0.05±0.01^{a}	0.04±0.01^{b}	0.04±0.01^{b}	0.05±0.01^{a}
Omega 3	0.64±0.08^{ab}	0.60±0.03^{b}	0.59±0.02^{b}	0.64±0.01^{a}
Omega 6	9.91±0.72	10.14±0.39	10.01±0.33	10.33±0.85
∑ SFA	36.96±0.96^{bc}	36.41±1.08^{b}	38.47±1.02^{a}	37.79±0.86^{ac}
∑ MUFA	49.01±1.51	49.52±1.87	50.27±1.64	49.40±0.41
∑ PUFA	11.45±0.85	11.73±0.55	11.86±0.20	11.84±0.95

Note: Values are given as mean ± SD (standard deviation); n = 30; C = control group; E1, E2, E3 = experimental groups; $^{a, b}$ = means within the same row with different superscripts differ significantly (P≤0.05)

Effect of natural feed supplements (humic acid and humic acid in combination with garlic and oregano powder) on fatty acid composition of breast and thigh muscles are shown in Table 2 and Table 3.

In comparison with control group, FA composition of breast muscle (Table 2), 7 out of 15 fatty acids was affected (P≤0.05) by dietary supplementation with humic acid (E1), combination of humic acid with garlic powder (E2) and combination of humic acid with oregano powder (E3). Among SFA, the concentration of myristic acid (C14:0) and palmic acid (C16:0) increased in breast muscle (P≤0.05) when chickens were fed with combination humic acid plus garlic powder (E2; 1.28 g.100 g^{-1}) and humic acid (E1; 24.23 g.100 g^{-1}) and humic acid plus garlic powder (E2; 24.57 g.100 g^{-1}), respectively. Among MUFA, concentration of oleic acid (C18:1 cis) was enhanced (P≤0.05) in breast muscle obtained from chickens in E2 (42.68 g.100 g^{-1}) and was decreased (P≤0.05) in the case oleic acid in E3 (39.14 g.100 g^{-1}) and eicosenoic acid (C20:1 n-9) in E3 (0.45 g.100 g^{-1}). The PUFA content of breast muscle was reduced mainly in the form of arachidonic (C20:4 n-6) in E3 (1.42 g.100 g^{-1}), docosapentaenoic (C22:5 n-3) and docosahexaenoic acid (C22:6 n-3) in E1 and E2 (0.14, 0.04, and 0.14, 0.04 g.100 g^{-1}, respectively). Yet, total amounts of MUFA and PUFA in experimental groups remained unchanged (P>0.05) compared with control group but content of SFA was increased (P≤0.05) in E2 (38.47 g.100 g^{-1}) compared with control group (36.96 g.100 g^{-1}). Overall, only 8 (lauric, heptadecanoic, stearic, vaccenic, linoleic, CLA, α-linoleic and eicosapentaenoic acids) out of 15 FAs that were detected in broiler breast muscle not differed (P>0.05) in proportion among the dietary treatments and control group.

Regarding thigh muscle (Table 3) significant differences (P≤0.05) in individual FAs among the groups were detected in 7 out of 15 FAs. Feed supplements affected (P≤0.05) a few FA proportions compared with those in control. The reduction (P≤0.05) in SFA was in lauric acid (C12:0) in E1, E2 and E3 (0.07, 0.05, and 0.06 g.100 g^{-1}, respectively); and stearic acid (C18:0) in E3 (10.54 g.100 g^{-1}) and increase in myristic acid (C14:0) in E3 (1.35 g.100 g^{-1}) Among MUFA, content of oleic acid (C18:1 cis) was increased (P≤0.05) in thigh muscle of chickens fed with a diet containing humic acid (E1; 50.91 g.100 g^{-1}), vaccenic acid (C18:1 trans-11) in E3 (4.84 g.100 g^{-1}) and eicosenoic acid (C20:1 n-9) in E1, E2 and E3 (0.74, 0.86 and 0.78 g.100 g^{-1}, respectively) compared with control group. The significant increase (P≤0.05) compared with control group was also observed in PUFA proportions, only in α-linolenic acid (C18:3 n-3) in E2 (2.07 g.100 g^{-1}).

In addition, there was a decrease (P≤0.05) in total SFA in E2 (32.63 g.100 g^{-1}) compared with control group (34.20 g.100 g^{-1}). Furthermore, total MUFA was enhanced (P≤0.05) in thigh meat from chickens receiving humic acid (E1; 54.45 g.100 g^{-1}) and decreased in combination with humic acid plus oregano powder (E3; 51.29 g.100 g^{-1}) compared with control group (53.66 g.100 g^{-1}). We found decreasing (P≤0.05) of total PUFA content in E2 (7.49 g.100 g^{-1}) compared with control group (8.78 g.100 g^{-1}).

Comparing breast with thigh muscle, one unanticipated finding was that breast contained slightly higher amounts of total SFA (36.41 to 38.47% in breast vs. 32.63 to 34.20% in thigh). Besides, breast muscle was found to contain slightly lower proportion of total MUFA (49.01 to 50.27% in breast vs. 51.29 to 55.50% in thigh). Not surprisingly, breast muscle had higher percentage of total PUFA (11.45 to 11.86% in breast vs. 7.49 to 9.87% in thigh), which is in agreement with the results of **Shin *et al.* (2011)** or **Trembecká *et al.* (2016).**

Regarding the FA profile of chicken meat, total SFA, MUFA, and PUFA proportions (mean of groups) in breasts were similar to those in reference tables of **USDA Food Composition Databases (2015)** (37, 49 and 12 g.100 g^{-1} vs. 56, 69 and 42 g.100 g^{-1} of fresh meat, respectively), whereas in thighs (referred as a sum of thigh plus drumstick) they were much lower than those reported by **USDA Food Composition Databases (2015)** (34, 54 and 9 g.100 g^{-1} vs. 105, 144 and 9.6 g.100 g^{-1} of fresh meat, respectively). Such differences may be due to variations in analytical methodologies, diets, and animal breeds.

Milićević *et al.* (2014), who investigated the impact of chicken meat consumption on cardiovascular risk in the general population, reported that the main FA identified in both breast and drumstick muscles were oleic acid (C18:1 cis) ranged between 37.12 and 39.56 % in breast and between 38.13 and 39.89 % in thigh muscle. In breast muscle, the major SFA was palmitic acid (16:0) and ranged from 21.35 to 28.53 %; and the major PUFA was linoleic acid (C18:2 cis n-6) and varied from 10.26 to 24.85 %. The FA composition of drumstick muscle showed a slightly higher fraction of linoleic acid (C18:2 cis n-6) in comparison to palmitic acid (16:0) (22.22 to 23.03 % vs. 21.52 to 23.69 %, respectively), which is also consistent with our results.

Nonetheless, it is important to keep in mind that even though unsaturated FAs are desirable for human consumption, with increasing degrees of lipid unsaturation, their susceptibility to oxidation increases, which makes meat preservation more difficult **(Ladeira *et al.*, 2014),** while also in our study, the content of unsaturated fatty acids predominantly decreased in thighs and too breasts, except group E2 and E3 of breast muscle. **Azman et al. (2004)** reported content of total SFA in thigh muscle of Ross 308 after feeding with soybean oil 42.14 g.100g^{-1}, total MUFA content 29.66 g.100g^{-1} and total PUFA content 22.06 g.100g^{-1}, similar **Hrdinka *et al.* (1996)** obtained 48.24 g.100g^{-1} SFA, 40.68 g.100g^{-1} MUFA and 7.12 g.100g^{-1} PUFA in thigh muscle of birds fed with soybean oil, while in our experiment, the measured lower SFA content from 32.63 – 34.20 g.100g^{-1}, higher MUFA 51.29 – 55.50 g.100g^{-1} and PUFA content was 7.49 – 9.87 g.100g^{-1}.

Table 3 The fatty acids profile (g.100 g^{-1}) of chicken thigh muscle (mean±*SD*)

Fatty acid\Group	C	E1	E2	E3
Lauric (C12:0)	0.08±0.01[a]	0.07±0.01[c]	0.05±0.02[b]	0.06±0.01[bc]
Myristic (C14:0)	1.30±0.02[b]	1.29±0.02[b]	1.27±0.01[b]	1.35±0.01[a]
Palmic (C16:0)	24.44±0.11	24.42±0.11	24.36±0.13	24.35±0.20
Heptadecanoic (C17:0)	0.29±0.05	0.27±0.02	0.25±0.03	0.26±0.05
Stearic (C18:0)	11.04±0.11[a]	11.09±0.09[a]	11.07±0.09[a]	10.54±0.30[b]
Oleic (C18:1 cis)	47.65±2.28[b]	50.91±2.23[ac]	53.13±3.27[a]	43.75±7.39[bc]
Vaccenic (C18:1 trans-11)	4.58±0.09[b]	4.56±0.03[b]	4.53±0.06[b]	4.84±0.10[a]
Linoleic (C18:2 cis)	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
Conjugated Linoleic (C18:2 n-6)	0.13±0.02	0.12±0.01	0.11±0.01	0.12±0.01
α-Linolenic (C18:3 n-3)	1.97±0.02[b]	1.99±0.12[b]	2.07±0.15[a]	1.98±0.17[b]
Eicosenoic (C20:1 n-9)	0.61±0.07[b]	0.74±0.11[a]	0.86±0.14[a]	0.78±0.15[a]
Arachidonic (C20:4 n-6)	1.45±0.17	1.39±0.06	1.28±0.14	1.44±0.18
Eicosapentaenoic (C20:5 n-3)	0.08±0.02[ab]	0.07±0.01[b]	0.07±0.02[b]	0.10±0.02[a]
Docosapentaenoic (C22:5 n-3)	0.13±0.01	0.13±0.01	0.13±0.01	0.14±0.02
Docosahexaenoic (C22:6 n-3)	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01
Omega 3	0.60±0.04[b]	0.63±0.05[ab]	0.69±0.06[a]	0.65±0.04[a]
Omega 6	6.95±0.81[a]	5.84±0.57[b]	5.77±1.06[ab]	6.85±1.22[ab]
∑ SFA	34.20±1.50[a]	34.17±1.07[a]	32.63±0.83[b]	33.20±1.23[ab]
∑ MUFA	53.66±0.41[a]	54.45±0.58[b]	55.50±1.78[ab]	51.29±0.77[c]
∑ PUFA	8.78±0.86[ac]	7.87±0.87[bc]	7.49±1.03[b]	9.87±1.53[a]

Note: Values are given as mean ± SD (standard deviation); n = 30; C = control group; E1, E2, E3 = experimental groups; [a, b] = means within the same row with different superscripts differ significantly (P≤0.05)

Scaife *et al.* (1994), **Hrdinka *et al.* (1996)**, **Crespo and Esteve-Garcia (2001)** and **Choi *et al.* (2010)** documented that oleic acid is a major fatty acid of breast, which was confirmed by all groups in our experiment and similar tendency was also retained in the thigh muscle. **Disetlhe *et al.* (2019)**, who fed chickens with humic acid, found similarly as in our experiment, found increased levels for most PUFAs, and a similar tendency was also found in groups with supplement of garlic and oregano powder. We no found higher differences (P≥0.05) in the PUFA content of the breast muscle after the addition of humic acid and garlic or oregano powder, but in the thigh muscle were higher value (P≤0.05) in experimental group with supplementation of the humic acid and garlic powder than in the control as reported by **Disetlhe *et al.* (2019)**. In the study **Choi *et al.* (2010)** results show that 3 and 5% garlic powder or 3% garlic powder plus α-tocopherol supplementation to diets can effectively change the fatty acid composition by increasing or protecting the oxidation of unsaturated fatty acid and total unsaturated fatty acid because palmitic and oleic acids are the main fatty acids of the thigh muscle, what is comparable to our results. There are 2 possible reasons for this phenomenon in the effectiveness of this product: 1) reduction in unsaturated fatty acid and total unsaturated fatty acid using garlic powder and α-tocopherol is related to peroxide-scavenging enzyme activity, which could reduce unsaturated fatty acid and total unsaturated fatty acid oxidation and 2) some active components in the garlic powder and α-tocopherol may involve desaturase and elongase activities (**Kim *et al.*, 2005; Guo *et al.*, 2006**). Thus, in terms of beneficial health-related biological properties, it is evident that increasing unsaturated fatty acid and total unsaturated fatty acid contents through dietary manipulation (garlic powder and α-tocopherol) could hold promise for the health of consumers (**Shahidi, 1996; Belury, 2002**). An important acid in chicken meat is also eicosapentanoic acid, which is a precursor of eicosanoids (prostaglandins, tromxoxanes, prostacyclins and leukotrienes) and is important for brain function and vision (**Dyall and Michael-Titus, 2008**). Its value in breast muscle was increased only after the humic acid addition (E1) and in the thigh muscle after the addition of a humic acid and oregano powder combination. These types of fatty acids are important for human health because they are precursors to the biosynthesis of eicosanoids, which are considered an important bio regulator of many cellular metabolic processes, blood pressure and blood clotting, tissue growth and immune system modulation (**Mao *et al*., 2015**) We have confirmed opinion that an increase in n-3 PUFA, especially alfa-linoleic acid in the muscle may cause a substantial decrease in arachidonic acid because of the action of delta-6/5-desturase enzymes in the elongation and desaturation metabolism (**Nuernberg *et al*., 2004**). The variation of fatty acid compositions has profound effects on meat quality, because fatty acid composition determines the firmness/oiliness of adipose tissue and the oxidative stability of muscle, which in turn affects flavour and muscle colour. It is well known that high PUFA levels may produce alterations in meat flavour due to their susceptibility to oxidation and the production of unpleasant volatile components during cooking (**Wood *et al*., 1999**). The nutritional properties of meat are largely related to its fat content and its fatty acid composition. In this sense, long-chain n-3 fatty acids, such as C20:5 n-3 and C22:6 n-3 have beneficial health effects, such as reduction in the thrombotic tendency of blood, associated with lower coronary heart disease in humans (**EFSA, 2010**). Consumption of unsaturated fatty acid, highlighting the PUFA, has shown potential benefits to consumer's health. A higher consumption of saturated fatty acid (SFA) than PUFA associated with the consumption imbalance of n-6/n-3 fatty acid, have been correlated to cardiovascular disease, cancer, inflammatory and auto¬immune diseases (**Simopoulos 2004; Wood *et al*., 2004**). The diet possibly has a significant influence on meat composition, especially broiler chickens. Thus, studies have reported the influence of an animal's diet on fatty acid profile of chicken meat (**Bonoli *et al*. 2007; Gatrell *et al*. 2015; Nkukwana *et al*. 2014; Rymer *et al*., 2011; Sun *et al*. 2012**).

CONCLUSION

The addition of garlic and oregano powder in combination with humic acids can affect the fatty acid profile of chicken meat. In thigh muscle, tested supplements decreased SFA content in experimental groups compared to the control group and increased the MUFA content in group with addition of humic acid or combination of humic acid and garlic powder. The PUFA content of the thigh muscle was increased only after the addition of the humic acids with oregano powder compared to the control. The effects of the tested supplements have not been unequivocally confirmed in all fatty acids; therefore we recommend further review to verify their effectiveness.

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