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THE AMINO ACID PROFILE AFTER ADDITION OF HUMIC ACIDS AND PHYTOBIOTICS INTO DIET OF BROILER CHICKEN

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ABSTRACT

The aim of the study was analysed the effect of humic acids separately and humic acids in combination with phytobiotic as garlic and oregano powder on amino acid (AA) profile of the most valuable parts of Ross 308 chicken. 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 AA profiles of breast and thigh samples were determined. In comparison with control group, 6 out of 10 AA was significantly affected ($p \leq 0.05$) by used dietary supplementation – Met, Cys and His in thigh and Leu, Phe, His and Arg in breast muscle. AA composition of breast muscle was positively affected mainly by humic acids and 10% oregano powder supplementation (E3), while thigh muscle by humic acids and 20% garlic powder (E2). The highest obtained AA in breast muscle was Leu (2.02 g.100 g^{-1}) in E3 group and thigh muscle His (1.15 g.100 g^{-1}) in E2 group ($p \leq 0.05$). In conclusion, humic acids and 10% oregano powder supplementation (E3) elicited to the best AA profile of chicken breast muscle but also the worst AA profile in thigh muscle so the effect of such a supplementation is disputable. On the other hand, humic acids and 20% garlic powder supplementation resulted into slight increase of AA in both breast and thigh muscle (E2).

Keywords: broiler chicken; amino acid; humic acid; garlic; oregano

INTRODUCTION

Meat and meat products are generally an important source of protein. Proteins are naturally occurring complex nitrogenous compounds and the percentage of the meat protein component varies considerably between different types of meat **(Marangoni et al., 2015)**. Average protein content in chicken meat is about 20 % and more than 40 % of AAs are essential ones **(Kim et al., 2017)**. Chicken meat offers all the EAAs and is characterized by a high content of lysine, leucine, aspartic acid and glutamic acid **(Sales and Hayes 1996; Soriano-Santos 2010; Ramane et al., 2011)**.

Although commercial feed additive containing antibiotics can increase productivity, antibiotic residues in meats might have negative effects on human health when meats were consumed **(Barton and Hart 2001; Khaksefidi and Rahimi, 2005)**. Thus, it needs alternative feed additives, which are safer, drug-free residue and to meet consumer demand. The possibility of using new natural materials including oregano, garlic and humic asids in diet of poultry is being researched **(Haščík et al., 2017; Kalafova et al., 2018)**. Many natural substances have been shown to express positive effects on growth performance and different health parameters in animals **(Hong et al., 2012; Thiamhirunsopit et al., 2014)**.

Many of plants and herbs can be used to improve the meat quality **(Liu et al., 2008)**, because of development of antibiotic resistance and their banned in the European Union since 2006. In addition, they consider the other alternative feed supplements, replacing the use of antibiotics in chicken nutrition. Probiotics and bee products **(Trembecká et al., 2017)**, organic acids, prebiotics, symbiotics, enzymes, organic minerals **(Fulton et al., 2002)** and plant essential oils **(Cross et al., 2007)** have been tested as suitable substitutes.

Plants and plant products, commonly referred to as phytobiotics, are natural, nontoxic, residue free, and readily available. Therefore, they are a suitable alternative to growth antibiotics. They have several effects on animals, such as appetite stimulation, increase digestive secretion, have immunostimulatory, bactericidal, antiviral and antioxidant effects **(Hashemi and Davoodi, 2011; Giannenas et al., 2013)**. Specially the medicinal activities of some natural plants as garlic (*Allium sativum* L.), thyme

(*Thymus vulgaris*), oregano (*Origanum vulgare*) and basil (*Ocimum basilicum*) are well known and documented **(Kostadinović, 2013)**.

Humates, one of the potential substances alternative to antibiotics in the diet of poultry, are formed from decayed plant matter with the aid of living bacteria in the soil. Humates include humus, humic acid, fulvic acid, ulmic acid, and trace minerals. Humic acid is a natural organic acid and has been shown to influence digestion, immune response and general performance of broilers **(Ozturk et al., 2012)**. The humic acids inclusion in broiler diets may stimulate changes in digestion dynamics, assimilation of nutrients and meat metabolite profiling, resulting in desirable meat compositional and organoleptic physiognomic quality **(Ozbey and Esen, 2007)**.

In chicken and pork, humic acid inclusion in diets was observed to desirably modify meat colour mainly due to accelerated myoglobin synthesis **(Wang et al., 2008; Ozturk et al., 2012)**. Moreover, in pork, humic acid was observed to have an effect of increasing the fat marbling values and to reduce back fat thickness, probably due its influence on protein and lipid distribution **(Wang et al., 2008)**. From the results of **Disetlhe et al. (2019)**, it can be concluded that the inclusion of enzymes and potassium humate in canola-based broiler diets had beneficial effects on the carcass and meat quality parameters in terms of breast weights, water holding capacity and colour.

Oregano (*Origanum vulgare* L.) is generaly an aromatic herb used to improve organoleptic characteristics of foods. Also it is a natural, less toxic, residue free feed supplement for poultry when compared to other synthetic ingredients. Its oil contains key bioactive components, including as thymol and carvacrol **(Figiel et al., 2010; Alagawany, et al., 2018)**. These phenolic compounds have an antimicrobial, antioxidant, antiviral, immunomodulatory and antiparasitic effect. The potential advantages of utilising oregano extract, in poultry diets include improved feed intake and feed conversion, enhanced digestion, expanded productive performance, down-regulated disease incidence and economic losses **(Arcila-Lozano et al., 2004; Fasseas et al., 2007)** and reduce conventional antibiotics use **(Symeon et al., 2010)**. From the available literature, average inclusions of oregano essential oil up to 600 mg/kg in broiler diets increased body weight gain. Using 1% oregano oil in broiler diets improved feed conversion ratio and feed utilisation **(Alagawany, et al., 2018)**. But, chemical composition of oregano oil may vary due to weather, season of year, harvest cycle, process of extraction and crop location **(Baydar et al., 2004; Ortega-Nieblas et al., 2011)**.

Garlic (*Allium sativum* L.) is widely used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases **(Javandel et al., 2008)**. Garlic is today use for a variety of reasons, it has anti-microbial, anti-bacterial, anti-inflammatory effects etc. **(Mansoub, 2011)**. Garlic was reported as a natural feed additive, it has improved broiler chickens growth and feed conversion **(Stanaćev et al., 2012)**. The major active ingredients in garlic are allicin, ajoene, diakyl polysulphides, s-allylcysteine etc which may be responsible for the various properties of garlic **(Canogullari et al., 2010)**. Garlic has been found to lower serum, liver and tissue cholesterol **(Stanaćev et al., 2012)**. In broilers, it was reported that garlic as a natural feed additive, improved broiler growth and feed conversion ratio, and decreased mortality rate **(Tollba and Hassan, 2003; Puvača et al., 2013)**. **Suriya et al. (2012)** suggested that inclusion of 0.5% garlic may have the potential to be an alternative to antibiotic growth promoter for broiler chicken.

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 amino acid (AA) profile of the most valuable carcass parts of Ross 308 chickens.

Scientific hypothesis

We are expecting the significant rise of AA content in breast and thigh muscle of broiler chicken after addition of humic acids, garlic and oregano into their diet.

MATERIAL AND METHODOLOGY

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 (1st – 21st days of age), grower (22nd – 35th days of age), and finisher (36th – 42nd 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 humic 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 AA composition. In breast and thigh muscles, measurements were made of the content of the EAAs (valine, leucine, isoleucine, phenylalanine, threonine, lysine, methionine) and NEAAs (histidine, arginine, cysteine) using an automatic amino acid analyzer AAA 400 (INGOS Prague, Czech Republic). This works on the principle of ionic exchange chromatography with post-column ninhydrin derivatization, based on procedure previously described by Moore and Stein (1963) approved by Bulletin of the Ministry of Agriculture and Rural Development of the Slovak Republic **(MARD SR, 2004)**. Post-column ninhydrin derivatization has become the official standard in recent years. There has been some methodological advancement, but the technique is still used very widespread. Total amino acid content of meat was determined by acid hydrolysis of proteins. Sulphur amino acids are first oxidized to a stable oxidized derivative, and then acid hydrolysed. Tryptophan was not determined because of its decomposition during acid hydrolysis of proteins. The resultant values of amino acids were re-calculated to 100% dry matter and expressed as g AA content per 100 g muscle.

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	34.50	34.50	36.32
Maize	35.50	40.50	37.50
Soybean meal (48% N)	21.40	18.80	20.00
Fish meal (71% N)	3.70	1.90	-
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	13.52	14.20	14.92
Fibre	30.17	29.92	30.53
Crude protein	210.73	190.41	170.54
MEN (MJ.kg^{-1})	12.15	12.04	12.41
Ash	24.22	19.92	38.47
Ca	8.16	7.28	7.38
P	6.77	5.71	6.01
Na	1.68	1.75	1.74

Note: *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; cobalamin 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, garlic and oregano into their diet, which was aimed at amino acid composition of breast and thigh muscle are presented into Table 2 and Table 3. The method used allowed measurement of 7 dispensable amino acids (EAA) and 3 indispensable amino acids (NEAA) in the muscles. The most abundant AAs (in decreasing order) in both breast and thigh muscles were Lys, followed by Leu, Arg, His, Thr and Phe.

Four AAs (Leu, Phe, His, Arg) in breast muscle were significantly 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) in comparison with control group (C). Their concentration in C group was Leu 1.94 g.100 g^{-1}, Phe 1.00 g.100 g^{-1}, His 1.12 g.100 g^{-1} and Arg 1.56 g.100 g^{-1}. Among EAAs, the concentration of Leu, respectively Phe has sligthly increased ($p \leq 0.05$) when chickens were fed with combination humic acid plus garlic powder (E2; 1.97 g.100 g^{-1} resp. 1.02 g.100 g^{-1}) and humic acid plus oregano leaf powder (E3; 2.02 g.100 g^{-1}, resp. 1.05 g.100 g^{-1}).

Table 2 Effect of natural feed supplements on amino acid composition of chicken breast muscle

Parameter\ Group	C	E1	E2	E3	*p*-value
			EAA		
Thr	1.09 ±0.10	1.02 ±0.12	1.11 ±0.10	1.13 ±0.08	0.079
Val	1.04 ±0.09	0.98 ±0.08	1.04 ±0.08	1.08 ±0.06	0.052
Met	0.79 ±0.08	0.74 ±0.08	0.79 ±0.06	0.82 ±0.06	0.066
Ile	0.95 ±0.12	0.89 ±0.10	0.97 ±0.09	1.00 ±0.07	0.051
Leu	1.94 $\pm0.22^{ab}$	1.81 $\pm0.20^{b}$	1.97 $\pm0.16^{ab}$	2.02 $\pm0.14^{a}$	0.049
Phe	1.00 $\pm0.11^{ab}$	0.94 $\pm0.10^{b}$	1.02 $\pm0.08^{ab}$	1.05 $\pm0.07^{a}$	0.045
Lys	2.10 ±0.25	1.95 ±0.22	2.13 ±0.18	2.19 ±0.15	0.051
			NEAA		
Cys	0.36 ±0.02	0.33 ±0.04	0.36 ±0.03	0.36 ±0.02	0.111
His	1.12 $\pm0.14^{ab}$	1.01 $\pm0.14^{b}$	1.13 $\pm0.12^{ab}$	1.19 $\pm0.09^{a}$	0.029
Arg	1.56 $\pm0.18^{ab}$	1.45 $\pm0.16^{b}$	1.58 $\pm0.13^{ab}$	1.63 $\pm0.11^{a}$	0.048

Note: Amino acids are expressed on a dry matter basis (g.100 g^{-1}). Values are given as mean ± *SD* (standard deviation); *n* = 50; C = control group; E1-E3 = experimental groups; $^{a-b}$ = means within the same row with different superscripts differ significantly ($p \leq 0.05$); EAA = essential amino acid; NEAA = non-essential amino acid; Thr = threonine; Val = valine; Met = methionine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine; Lys = lysine; Cys = cysteine; His = histidine; Arg = arginine.

Table 3 Effect of natural feed supplements on amino acid composition of chicken thigh muscle

Parameter\ Group	C	E1	E2	E3	*p*-value
			EAA		
Thr	1.09 ±0.01	1.08 ±0.04	1.12 ±0.09	1.05 ±0.08	0.167
Val	1.04 ±0.01	1.02 ±0.03	1.04 ±0.08	1.00 ±0.06	0.173
Met	0.79 $\pm0.01^{b}$	0.81 $\pm0.04^{ab}$	0.87 $\pm0.05^{a}$	0.78 $\pm0.05^{b}$	0.011
Ile	0.97 ±0.01	0.98 ±0.07	1.02 ±0.07	0.99 ±0.09	0.138
Leu	1.97 ±0.02	1.99 ±0.12	2.07 ±0.15	1.98 ±0.17	0.175
Phe	1.01 ±0.01	1.03 ±0.06	1.07 ±0.08	1.02 ±0.09	0.166
Lys	2.13 ±0.02	2.16 ±0.14	2.26 ±0.17	2.15 ±0.20	0.107
			NEAA		
Cys	0.35 $\pm0.01^{b}$	0.36 $\pm0.01^{b}$	0.39 $\pm0.03^{a}$	0.33 ± 0.01^{c}	0.001
His	1.15 $\pm0.01^{a}$	1.13 $\pm0.03^{a}$	1.15 $\pm0.08^{a}$	1.04 ± 0.06^{b}	0.009
Arg	1.58 ±0.02	1.60 ±0.11	1.67 ±0.12	1.60±0.15	0.127

Notes: Amino acids are expressed on a dry matter basis (g.100 g^{-1}). Values are given as mean ± *SD* (standard deviation); *n* = 50; C = control group; E1-E3 = experimental groups; $^{a-c}$ = means within the same row with different superscripts differ significantly ($p \leq 0.05$); EAA = essential amino acid; NEAA = non-essential amino acid; Thr = threonine; Val = valine; Met = methionine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine; Lys = lysine; Cys = cysteine; His = histidine; Arg = arginine.

The content of mentioned AAs has decreased ($p \leq 0.05$) after addition of humic acids (E1; 1.81 g.100 g^{-1}, resp. 0.94 g.100 g^{-1}) in comparison with control group. Among NEAAs, amount of His, resp. Arg was positively affected ($p \leq 0.05$) in E2 and E3 groups compared to C group. Their concentration was 1.12 g.100 g^{-1}, resp. 1.58 g.100 g^{-1} in E2 group and 1.19 g.100 g^{-1}, resp. 1.63 g.100 g^{-1}.

Supplementation with humic acids alone led to slight decerase in His, resp. Arg to 1.01 g.100 g^{-1}, resp. 1.45 g.100 g^{-1}. Other AAs in breast muscle were affected in the same way, but not significantly ($p \geq 0.05$).

In thigh muscle, three AAs (Met, Cys, His) were significantly affected ($p \leq 0.05$) by used dietary supplementations. Their concentration in C group was Met 0.79 g.100 g^{-1}, Cys 0.35 g.100 g^{-1} and His 1.15 g.100 g^{-1}.

Among EAAs, the concentration of Met, respectively, has sligthly increased ($p \leq 0.05$) when chickens were fed with combination humic acids (E1; 0.81 g.100 g^{-1}) and humic acid plus garlic powder (E2; 0.87 g.100 g^{-1}). The content of Met has slightly decreased ($p \leq 0.05$) after addition of humic acids (E3; 0.78 g.100 g^{-1}) in comparison with C group. Among NEAAs, amount of Cys was very gently increased ($p \leq 0.05$) in E1 (0.36 g.100 g^{-1}) and E2 (0.39 g.100 g^{-1}) and decreased in E3 (0.33 g.100 g^{-1}) groups compared to group C. On the other hand, Arg was not positively affected by any of used supplementation and was even decreased by humic acids plus oregano powder supplementation (E3; 1.04 g.100 g^{-1}). As in the breast muscles, other AAs in thigh muscle were affected similarly, but not significantly ($p \geq 0.05$).

The most abundant AAs (in decreasing order) in both breast and thigh muscles were Lys, followed by Leu, Arg, His, Thr and Phe. The lowest obtained AA was Cys in both breast and thigh muscle. Comparing breast with thigh muscle, the breast was found to contain higher amounts of all the EAAs and NEAAs detected in chicken meat, either the essential or the non-essential ones, which contrast with results reported by **Chae et al. (2012)**, who observed higher AAs contents in chicken thigh compared with breast muscle. Similarly, higher values of AAs in chicken meat were found by **Sharipova et al. (2017)** except Lys and Tyr. According to **Strakova et al. (2006)** the general levels of individual amino acids in breast muscles of broiler chickens varied from 1.9 (Pro) to 11.0 (Glu) g.100 g^{-1}, while in thigh muscles ranged from 1.4 (Met) to 9.3 (Glu) g. 100 g^{-1}. The total sum of amino acids in broiler chickens was 78.7 g.100 g^{-1} in the breast muscles (essential AAs – 42.5 g.100 g^{-1} and non-essential AAs – 36.1 g.100 g^{-1}) and 59.1 g.100 g^{-1} in the case of thigh muscles (essential AAs – 30.1 g.100 g^{-1} and non-essential AAs – 29.2 g.100 g^{-1}).

When comparing between dietary groups, contents of most EAAs were the highest in experimental group E3 in breast muscle, with one expectation (Cys) and the lowest in E1 group. On the other hand, the breast muscle was affected different by our supplementation – E3 had the lowest AA content and the best affected group was E2.

Amino acids are generally seen as main precursors of flavour substances. In particular, Glu was shown to have considerable effect on taste of chicken meat. In addition to Glu, free aromatic AAs, such as Phe and Tyr, also play an important role in enhancing the savoury or umami taste at sub-threshold concentrations in the presence of salt and free acidic AAs **(Wattanachant et al., 2004; Huang et al., 2011)**. Other AAs, such as Cys, Gly, Asp, Arg, and Ala, are also considered the flavour-related AAs **(Liu et al., 2008)**. Independently from these results, it is important to emphasize the way of heat treatment to AA composition. From the results of **Shehab (2016)** it is clear that any heat treatment of fresh thighs or breasts causes some reduction in all amino acids. Samples from chicken breasts and thighs cooked under pressure retained the highest percentage of total, essential and non-essential amino acids. Methods such as ordinary cooking, oven and frying followed. In particular, their loss in juice separated during cooking as well as thermal decomposition may be responsible for the reduction of the amino acid content.

CONCLUSION

The addition of garlic and oregano powder in combination with humic acids can affect the AA profile of chicken meat. In breast muscle, supplementation with humic acids plus garlic povder and also oregano increased content of all AA. Unfortunately, humic acids alone decreased AA content in experimental groups compared to the control group. The AA content of the thigh muscle was increased only after the addition of the humic acids with garlic powder compared to the control. The effects of the tested supplements may positively influence AA content; however, we recommend further review to verify their effectiveness.

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