Multiple factorial analysis of physicochemical and organoleptic properties of breast and thigh meat of broilers fed a diet supplemented with humic substances

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ABSTRACT   Multiple factor analysis was used for the examination of meat quality of broilers, the diet of which was supplemented with 0.8 and 1.0% addition of humic substances (**HS**). One hundred fifty COBB 500 one-day-old male broilers chicks were randomly divided into 3 different groups: one control and 2 experimental (n = 50), and they were fattened for 35 D. Subsequently, the meat quality was analyzed and defined by physico-chemical and sensory quality, supplemented with analysis by instrumental methods. We observed changes in dry matter, fat, water, and protein content in experimental samples of breast and thigh meat ($P < 0.001$). In both experimental groups, the concentration of phosphates and pH decreased in breast meat ($P < 0.001$) and in thigh meat ($P < 0.05$). The smell of experimental chicken breast meat samples after cooking was evaluated by a sensory panel, which scored a higher point score than that of the control group ($P < 0.05$). Sensory evaluation of taste indicated a positive response with respect to the perception of meat quality in relation to a greater supplementation of HS in the diet. Thigh meat samples showed a variable extent of water loss after cooking, but lower values of water loss were generally obtained from thigh meat samples of poultry fed with higher HS supplementation, than in chicken breast meat samples. Significant differences in evaluated variables between both experimental groups were not observed. The color of breast meat samples changed, when considering the variables of lightness and redness, with the addition of 1% HS ($P < 0.05$). The main advantage of the breast meat of broilers fed a diet supplemented with HS was observed in the final meat quality, which was positively affected by increased protein and decreased fat content. Because of its nutritional composition, it can be considered to be rather a valuable type of meat in human nutrition than ordinarily.

Key words: meat, quality, humic substances, statistics

INTRODUCTION

Poultry meat production depends on feed as the one of the main factors. Parameters that affect meat quality are complex and occur throughout the production chain (Vašková et al., 2015). Supplying poultry meat of adequate quantity and quality to meet public demand is a major objective of the broiler industry (Esenbuga et al., 2008). From the perspective of the consumer,

poultry is a very attractive and essential element in the human diet because of its nutritional, dietetic, and sensory properties, and its rapid culinary preparation (Vašková et al., 2018).

The development of newer, efficacious techniques to enhance the production and health of poultry is vital for the modern, ever-evolving poultry meat industry (Edmonds et al., 2014). Diet composition influences carcass yield and quality. Age, sex, strain, diet, intramuscular fat, meat moisture content, preslaughter conditions, and processing variables alter broiler meat color (Esenbuga et al., 2008). Color, pH, and water-holding capacity are important characteristics of meat quality that can affect consumer preferences (Ozturk et al., 2012).

Humic substances (**HS**) are a product of the decomposition of organic matter, particularly that of plant origin

(Arif et al., 2018) and soil constituents (Rath et al., 2006). HS, as a growth-promoting agent, exerts a multitude of positive health effects and provides a broad range of nutritional benefits for domestic animals (Ozturk et al., 2012). The use of humates in animal nutrition as feed additives is a recent concept in the industry, and, in particular, humates have been used to combat the after-effects of disturbances of the digestive system, such as diarrhea and malnutrition, as a component of replacement therapy (Esenbuga et al., 2008; Arif et al., 2018). Better feed efficiency with lower mortality rates have been reported in broilers utilizing humates (Arif et al., 2018).

The effects of different levels of the HS in poultry production have been studied in recent works (Kocabağli et al., 2002; Ceylan et al., 2003; Karaoglu et al., 2004; Rath et al., 2006; Ozturk et al., 2010, 2012; Marcinčáková et al., 2015; Vašková et al., 2015). Ozturk et al., (2010) evaluated the effects of different doses of HS supplementation, provided through drinking water, on the following parameters: growth performance, characteristics of the carcass and gut, and chemical composition of samples from broilers. Humates administered in food or water promote poultry growth (Žatko et al., 2014). A positive effect of HS was also found in feed conversion of broiler poultry (Kocabağli et al., 2002) and also on meat quality (Ozturk et al., 2010).

However, any studies about the effect of broilers fed supplementation with HS on meat quality, supportive meat sensory evaluation, or instrumental analyses of texture and colorimetry remain yet to be published. This experiment was primarily designed to examine the effect of the supplementation of dietary HS on physical and chemical variables, as well as on the sensory quality of breast and thigh meat, including instrumental color and textural parameters, by multiple factorial analysis.

MATERIAL AND METHODS

The animal protocol for this research was approved by the Ethical Committee for Animal care and Use of University of Veterinary Medicine and Pharmacy in Košice (Slovak Republic). The experiment was carried out in accordance with the "European Directive on the protection of vertebrate animals used for experimental and other scientific purposes" (European Commission, 2010) and with the consent of the State Veterinary and Food Administration of the Slovak Republic No. 3040/14-221 in the premises of Clinic for birds, exotic, and free living animals of the University of Veterinary Medicine and Pharmacy in Košice (Slovak Republic).

Experimental Chickens Groups

For the trial, 150 one-day-old COBB 500 (*Gallus gallus domesticus*) sexed male broiler chickens purchased from supplier (Mach Hydina Budmerice, Hydina

Slovensko, Ltd., Slovak Republic) were randomly divided into one control group (C) and 2 experimental groups, each group consisting of 50 pieces, with 3 replications (16, 17, 17 per pen). Chickens of the first experimental group were fed with a diet enriched with 0.8% supplementation of HS (HS0.8), and those of the second experimental group were fed a diet enriched with 1.0% (HS1.0) supplementation of HS. The HS were adherenced to the pellets of the basic feed mixtures, which were used for fattening: starter diet during the first 10 D of fattening; growing diet from day 11 to 28; final diet from day 29 to 35. The composition of HS and basic feed mixtures are presented in Tables 1 and 2. Control group of animals was fed with basic feed mixtures (starter, grower, and finisher) without supplementation of HS. The main components of feed mixtures were wheat, corn, soybean meal, rapeseed cake, and sunflower meal. Conventional feed mixtures were sampled for analyses. In feed samples, nutrient content was analyzed using standard methods and procedures according to the Commission Regulation (EC) no. 691/2013 (European Commission, 2013), which laid down the approved methods of sampling and analysis for the official control of feed. Broilers were reared on deep litter under controlled conditions. During the whole time of fattening, the light and temperature regime was monitored (COBB Broiler Management Guide, 2013). During the trial, a 24-h light regime was set on the first day, and this was subsequently decreased to 18-h. The ambient temperature was gradually lowered from an initial level of 33°C (day 1) to 21°C (day 24), and the ambient humidity was maintained at approximately 70%. The animals had access to water and feed *ad libitum* during fattening. Neither clinical symptoms of disease nor abnormal mortality were observed during the fattening period. Body weight of individual broiler chicks was measured at weekly intervals, feed consumption was recorded each day, and feed conversion ratio was calculated at the end of experiment. The carcass yield was determined as a proportion of the body weight before slaughter and after evisceration.

Table 1. The composition of natural humic substances according to Jaďuttová et al. (2019).

Components	value
Humic acids in dry matter (%)	min. 65.00
Free humic acids in dry matter (%)	min. 60.00
Fulvic acids (%)	min. 5.00
Ca (mg/kg)	42,278
Mg (mg/kg)	5,111
Fe (mg/kg)	19,046
Cu (mg/kg)	15.00
Zn (mg/kg)	37.00
Mn (mg/kg)	142.00
Co (mg/kg)	1.24
Se (mg/kg)	1.67
V (mg/kg)	42.10
Mo (mg/kg)	2.70

Abbreviations: Ca, calcium; Co, cobalt; Cu, cuprum; Fe, ferrum; Mg, magnesium; Mn, manganese; Mo, molybdenum; Se, selenium; V, vanadium; Zn, zinc.

Table 2. Composition of broiler diets.

Ingredient	Starter	Grower	Finisher
Corn (%)	37.50	39.00	41.00
Wheat (%)	26.00	28.50	30.00
Soybean meal (%)	23.00	20.00	17.00
Dehulled sunflower meal (%)	2.90	2.80	2.70
Fish meal (%)	3.00	-	-
Rapeseed cake (%)	2.70	4.70	4.40
Lard (%)	2.00	1.85	1.90
Limestone (%)	0.62	0.80	0,70
Monocalcium Phosphate (%)	1.65	1.65	1.55
Salt (%)	0.13	0.20	0.25
Premix of vitamins and minerals[1] (%)	0.50	0.50	0.50
Composition (analyzed)			
Dry matter (%)	90.20	89.40	89.50
Crude protein (%)	20.60	18.10	17.47
Crude fat (%)	4.41	3.74	5.11
Dietary fiber (%)	2.80	3.50	4.61
Starch (%)	39.00	41.60	43.20
Ash (%)	5.20	4.10	3.49
Ca (%)	0.94	0.88	0.84
Mg (%)	0.10	0.09	0.10
Na (%)	0.14	0.15	0.12
K (%)	0.80	0.64	0.61
P (%)	0.53	0.50	0.48
Cu (ppm)	16.30	10.20	11.90
Zn (ppm)	108.50	99.35	97.70
Mn (ppm)	92.30	108.19	85.90
ME (MJ/kg)	11.81	13.71	13.75

Abbreviations: CP, crude protein; ME, metabolizable energy.

[1]Supplied per kg of basal diet: vitamin A—12,500 IU; vitamin D3—4,000 IU; vitamin E—40 mg; vitamin K3—3 mg; I—1 mg; Co—0.7 mg, K—8.6 g; Cl$^-$—2 g; Cu—20.0 mg; Fe—60 mg; Zn—80 mg; Mn—90 mg; Se—0.35 mg.

Physicochemical Analysis of Meat Samples

AOAC (1990) methods were used for moisture, dry matter, protein, and fat content of breast and thigh samples. Water loss after cooking was measured by dividing the percentage of the weight loss in the meat sample by the difference between raw weight and cooked weight. The results were expressed as percentage over fresh matter basis. The pH of meat samples was analyzed with a digital inoLab pH 340i meter (Wissenschaftlich-Technische Werkstätten, Germany). The content of lactic acid and phosphates were determined according to the method executed by Mačanga et al. (2011), which utilized the capillary electrophoretic separation system Type EA102 (Villa Labeco, Slovak Republic), and results were subsequently processed by ITPPro 32 (Kas-Comp, Bratislava, Slovakia).

Color Measurement

The color of meat samples was quantitatively measured by a Chroma meter CR-410 (Minolta, Osaka, Japan) using CIE (International Commision on Illumination) values (McLaren, 1976). For the measurement of colorimetric data, Chroma meter CR-410 (measurement area ∅ 50 mm, illuminance D65, standard observer angle 2°, Konica Minolta, Sensing, Inc.) and Color Data Software CM-S100w SpectraMagic NX (Konica Minolta Sensing Inc.) were used. The Chroma meter was standardized using a white standard plate (CR-A43, Konica Minolta). The results reported are average values of the 3 measurements.

Instrumental Texture Analysis

The texture profile analysis was performed using TA-XT Plus Texture Analyzer (Stable Micro System, Surrey, UK), according to Čurlej et al. (2015) with modifications. The samples were examined using a Stable Micro Systems Type (version 9.0). The setting was adjusted to compression test mode, pretest speed of 2 mm s^{-1}, a test speed of 2 mm s^{-1}, and a posttest speed of 10 mm s^{-1}. All the samples were compressed using a cylindrical probe to a target distance of 20 mm. The measurements of raw meat breast samples were made at ambient temperature (20°C ± 2°C) and again after cooking at 75°C for 10 min in water bath and allowing the sample to cool down to ambient temperature. The obtained texture profiles were used to measure the instrumental maximum peak positive force (hardness) of meat samples.

Sensory Analysis

Samples were stored at 4°C ± 2°C until use. The meat samples were portioned into 25 × 25 × 25 mm cubes using a wire slicer, each cube weighed approximately 25 g, and they were served in white plastic dishes and coded with 3-digit random numbers. Samples were served at a temperature of 20°C ± 2°C. Mineral water was provided for mouth-rinsing. The sensory evaluation was carried out in a standardized sensory laboratory (ISO 8589, 2014) built in the Institute of Postgraduate Education of Veterinary Medicine in Košice (Slovakia). The sensory evaluation was performed by a panel consisting of staff from the University of Veterinary Medicine and Pharmacy in Košice (Slovakia). The panel consisted of 12 panelists, aged between 28 and 65 yr. All the assessors were trained in the sensory analysis of meat before the analysis. All assessors evaluated smell, taste, juiciness, brittleness, and overall acceptability of each sample on a 5-point protocol. The overall acceptability of the served samples were evaluated using a 5-point hedonic scale (1—dislike extremely, 2—dislike moderately, 3—neither like nor dislike, 4—like moderately, 5—like extremely).

Statistical Analysis

Data analysis was carried out via the R-statistics software (R. C. Team, 2017). ANOVA and Tukey test, for multiple comparison of means at a significance level of $P < 0.05$, were conducted. The effect of addition of HS in the diet of the 2 experimental groups was set as the main factor. Multiple factor analysis (**MFA**) was conducted in R–statistics software with "FactomineR" (Lê et al., 2008) and "factoextra" package (Kassambara and Mundt, 2007). MFA method visualized the results by 2 plots: correlation circle and graph of individuals. The correlation circle shows the relationship between analyzed variables, the quality of the representation of variables, and the correlation between variables and the 2 extracted dimensions. Positive correlated variables

are visualized on the plot together, whereas negative ones are positioned on opposite sides of the plot. The distance between variable points and the origin measures the quality of the variable on the factor map. The graph of individuals shows representations of individuals in which individuals are much closer than they have similar values for all variables in all groups.

RESULTS AND DISCUSSION

The results of body weight and calculated feed conversion ratio were reported previously by authors Jaďuttová et al. (2019). Both experimental groups (HS0.8 and HS 1.0) showed an nonsignificant increase in both body weight and weight gain, in comparison to the control group of broilers ($P > 0.05$). The highest mean body weight was recorded in the experimental group HS1.0. The feed consumption was similar in all groups during the fattening period ($P > 0.05$), but there was an improvement in the feed conversion ratio, particularly for the group fed with 1.0% addition of HS. No significant differences were recorded in the carcass yield ($P > 0.05$), but there was a significant increase in the yields of breast and thigh meats of broilers from experimental group HS1.0 in comparison to that of the control group ($P < 0.05$).

Table 3 shows the effect on breast and thigh meat samples obtained from broilers belonging to the experimental groups in relation to the addition of HS in their diet, which resulted in differences in the following measured parameters: dry matter, fat, water, proteins, phosphates, and pH ($P < 0.001$). Differences in water and protein content of breast meat samples were found between control and both experimental groups ($P < 0.001$). The fat content of breast meat decreased in relation to the addition of HS from $3.40 \pm 0.18\%$ in control group and 0.8% HS addition to 2.76 $\pm$ 0.01% and to 2.69 $\pm$ 0.05% with 1.0% HS addition in diet ($P < 0.001$). Addition of HS in chicken diet resulted in increased protein content of breast meat samples. However, the highest protein content in breast meat samples was observed in samples of HS0.8 experimental group. The increased HS supplementation (1.0%) resulted in increased protein content in breast meat samples in comparison with control, but it was not higher than the value obtained for the HS0.8 group. HS might increase the uptake of nitrogen, phosphorus, and other nutrients because of their chelating properties (Trckova et al., 2005). We assume that increased nitrogen intake resulted in an increase in total proteins in breast meat. Bai et al. (2013) discussed the effects of HS, whereby the presence of some biologically active substances in HS may be associated with, or have the ability to cause, the redistribution of proteins and lipids, thus leading to improved carcass traits (Wang et al., 2008). Increase of the phosphate content in experimental meat samples was confirmed only in thigh muscle. However, in both experimental groups, the content of phosphates in the breast meat samples was found to be lower than that for the breast meat samples belonging to the control group. We have not observed similar results in other experiments, and it is difficult to compare these results with other previous published studies. It will be necessary to study the impact of HS on different muscle tissues and on deeper parts of broiler meat. We can speculate that these differences in the phosphate content could be a result of the different chemical composition of the breast and thigh meat and variability in histological composition of muscle tissue. Barbut (2001) describe differences between broiler thigh and breast tissues, whereby thigh tissue has a high proportion of fibers similar to the red muscle fibers, whereas the breast meat is composed almost entirely of white fibers.

Table 3. The results of physicochemical analysis of breast and thigh meat samples (mean $\pm$ SD).

Physicochemical variables	Control	HS0.8	HS1.0	P-value
Breast meat samples				
Dry matter (%)	24.78 ± 0.13^b	26.21 ± 0.06^a	26.04 ± 0.45^a	$P < 0.001$
Fat (%)	3.40 ± 0.18^a	2.76 ± 0.01^b	2.69 ± 0.05^b	$P < 0.001$
Water (%)	75.22 ± 0.13^a	73.79 ± 0.06^b	73.96 ± 0.45^b	$P < 0.001$
Proteins (%)	22.02 ± 0.36^c	23.71 ± 0.21^a	23.01 ± 0.20^b	$P < 0.001$
Water loss after cooking (%)	31.12 ± 5.34	28.98 ± 1.36	29.31 ± 1.10	$P > 0.05$
Lactic acid (%)	1.77 ± 0.15	2.00 ± 0.36	1.82 ± 0.04	$P > 0.05$
Phosphates (%)	1.76 ± 0.18^a	0.90 ± 0.13^b	0.92 ± 0.07^b	$P < 0.001$
pH	5.96 ± 0.07^a	5.86 ± 0.05^b	5.80 ± 0.06^b	$P < 0.001$
Thigh meat samples				
Dry matter (%)	29.48 ± 0.29^b	30.73 ± 0.28^a	29.50 ± 0.10^b	$P < 0.001$
Fat (%)	11.29 ± 0.53^b	12.42 ± 0.29^a	11.04 ± 0.52^b	$P < 0.001$
Water (%)	70.52 ± 0.29^a	69.27 ± 0.28^b	70.50 ± 0.10^a	$P < 0.001$
Proteins (%)	18.36 ± 0.29^b	19.34 ± 0.27^a	18.41 ± 0.17^b	$P < 0.001$
Water loss after cooking (%)	31.62 ± 1.41^a	21.30 ± 3.96^b	20.96 ± 1.72^b	$P < 0.001$
Lactic acid (%)	1.00 ± 0.14	1.11 ± 0.09	0.97 ± 0.04	$P > 0.05$
Phosphates (%)	0.61 ± 0.06^b	$0.71 \pm 0.05^{a,b}$	0.73 ± 0.10^a	$P < 0.05$
pH	6.14 ± 0.09^a	$6.01 \pm 0.06^{a,b}$	5.99 ± 0.13^b	$P < 0.05$

Control, broilers fed with complete feed mixture; HS0.8, experimental group of broilers fed with diet enriched with 0.8% addition of humic substances; HS1.0, experimental group of broilers fed with diet enriched with 1.0% addition of humic substances.
[a–c]Means in row with a different superscript letter are statistically different (Tukey's, $P < 0.05$).

Table 4. The sensory evaluation results of breast and thigh meat samples (mean ± SD).

Sensory variables	Control	HS0.8	HS1.0	P-value
Breast meat samples				
Smell	3.17 ± 0.75^{b}	$3.83 \pm 1.17^{a,b}$	4.50 ± 0.55^{a}	$P < 0.05$
Taste	3.75 ± 0.61	4.17 ± 0.98	4.00 ± 0.63	$P > 0.05$
Juiciness	2.58 ± 0.49	3.83 ± 1.17	2.83 ± 0.98	$P > 0.05$
Brittleness	3.50 ± 1.05	3.67 ± 1.21	3.00 ± 0.63	$P > 0.05$
Overall acceptability	3.19 ± 0.19	3.88 ± 0.86	3.58 ± 0.38	$P > 0.05$
Thigh meat samples				
Smell	3.58 ± 0.92	3.17 ± 0.41	4.17 ± 0.75	$P > 0.05$
Taste	3.33 ± 0.52	3.83 ± 0.75	4.08 ± 0.66	$P > 0.05$
Juiciness	3.50 ± 1.05	4.17 ± 0.75	3.62 ± 0.87	$P > 0.05$
Brittleness	3.75 ± 0.76	3.83 ± 0.75	3.83 ± 0.75	$P > 0.05$
Overall acceptability	3.56 ± 0.27	3.71 ± 0.33	3.85 ± 0.12	$P > 0.05$

Control, broilers fed with complete feed mixture; HS0.8, experimental group of broilers fed with diet enriched with 0.8% addition of humic substances; HS1.0, experimental group of broilers fed with diet enriched with 1.0% addition of humic substances.

[a–c]Means in row with a different superscript letter are statistically different (Tukey's, $P < 0.05$).

We evaluated organoleptic characteristics of the breast meat and thigh meat samples of the experimental broiler chickens, after the cooking process in a water bath. The results of sensory evaluation are presented in Table 4. Assessors evaluated smell, taste, juiciness, brittleness, and overall acceptability on a 5-point scale. Differences in organoleptic properties of thigh meat samples were not observed ($P > 0.05$). However, we found a difference in the experimental groups when compared with the control group only with respect to the overall smell in breast samples. From a quantitative point of view, the score given to the meat of the experimental group HS1.0 by the evaluators was 1.42 times greater than that given to the control group ($P < 0.05$). The meat odor of the test group HS0.8 was also evaluated by a higher score (3.83 ± 1.17) vs. the control group (3.17 ± 0.75) ($P > 0.05$). Taste and juiciness in both experimental groups (HS0.8 and HS1.0) were evaluated in both breast and thigh meat samples with higher point score ($P > 0.05$) than control group. Although, when evaluated by the sensory panel, the overall sensory acceptability of breast and thigh meat samples of both experimental groups (HS0.8 and HS1.0) were granted higher points, in comparison to the points obtained by the control group. However, these differences were not significant ($P > 0.05$), which suggests that higher HS concentration (1.0%) does not improve these evaluated parameters. The HS may have the potential to improve the organoleptic properties of chicken meat. Currently, there are no existing studies that have investigated the impact of HS on the sensory quality of produced meat;

therefore, it is necessary to confirm this ability by further experiments. Improvement of organoleptic characteristics can also be caused by lower water loss during cooking, thus providing better juiciness and improved taste of evaluated meat.

The results of instrumental colorimetric and textural analysis of breast meat samples are presented in Table 5. Supplementation of HS in the chicken diet (1.0%) decreased the L* value in breast meat samples ($P < 0.05$). Color may also influence the pH of the poultry meat's colorimetric variables (Ozturk et al., 2012). In breast meat samples, differences in a* colorimetric value ($P < 0.05$) were also found. Esenbuga et al. (2008) conducted a color evaluation of thigh and breast muscles of the chickens fed a diet with addition of HS by demonstrating the changes in meat color. We observed an increase in colorimetric redness values (a*) in breast meat of the experimental groups, when compared with those of the control group ($P < 0.05$). However, regarding the redness values between both experimental groups HS0.8 and HS1.0, similar results were observed. Based on these observations, we can conclude that higher HS concentration in broilers fattening diet did not affect the redness of breast meat. Our results correspond to those obtained by Ozturk et al. (2012), who also observed an increased trend in a* values related to the addition of HS in broiler fattening diets. With respect to our results obtained from brightness measurement of samples, the addition of humic acids caused the muscle tissue of chicken breast meat samples to be darker. L* colorimetric value showed

Table 5. The results of instrumental colorimetric and textural analysis of breast meat samples (mean ± SD).

Variables	Control	HS0.8	HS1.0	P-value
L*(D65)	60.98 ± 2.02^{a}	$60.38 \pm 1.21^{a,b}$	58.10 ± 1.31^{b}	$P < 0.05$
a*(D65)	10.90 ± 1.12^{b}	12.27 ± 0.18^{a}	12.14 ± 0.81^{a}	$P < 0.05$
b*(D65)	11.78 ± 0.97	10.82 ± 2.72	11.21 ± 0.99	$P > 0.05$
C*(D65)	16.10 ± 0.53	16.47 ± 1.84	16.53 ± 1.24	$P > 0.05$
h*(D65)	47.25 ± 4.96	40.78 ± 6.82	42.66 ± 1.04	$P > 0.05$
Hardness before cooking (kg)	1.13 ± 0.22	1.30 ± 0.29	1.32 ± 0.23	$P > 0.05$
Hardness after cooking (kg)	1.78 ± 0.30	1.67 ± 0.27	1.66 ± 0.27	$P > 0.05$

Control, broilers fed with complete feed mixture; HS0.8, experimental group of broilers fed with diet enriched with 0.8% addition of humic substances; HS1.0, experimental group of broilers fed with diet enriched with 1.0% addition of humic substances.

[a–c]Means in row with a different superscript letter are statistically different (Tukey's, $P < 0.05$).

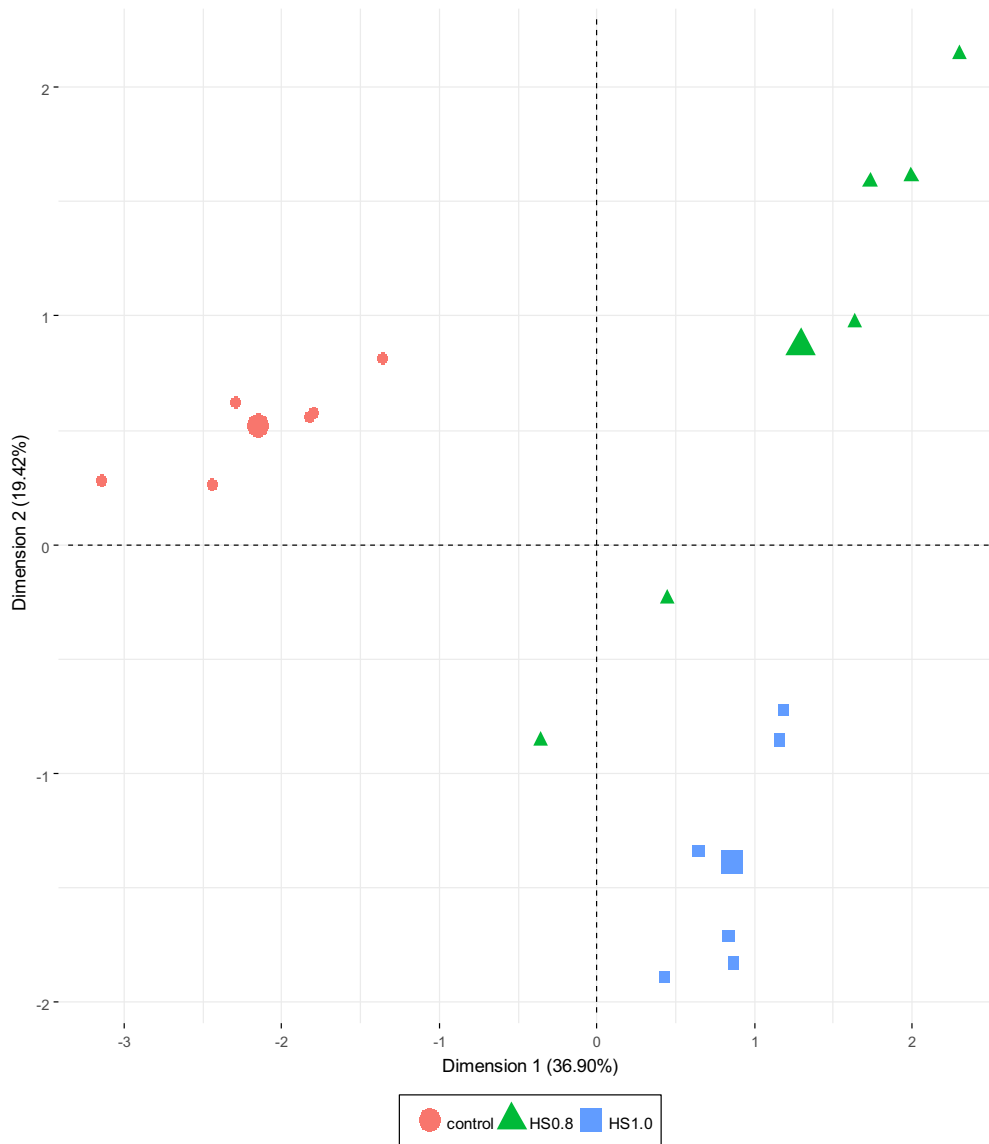The results of MFA analysis of breast meat samples: Individuals

Figure 1. The results of MFA analysis of breast meat samples: Individuals. Control, broilers fed with complete feed mixture; HS0.8, experimental group of broilers fed with diet enriched with 0.8% addition of humic substances; HS1.0, experimental group of broilers fed with diet enriched with 1.0% addition of humic substances. Abbreviation: MFA, multiple factor analysis.

a decrease in accordance with a higher addition of HS ($P < 0.001$). Ozturk et al. (2012) found a statistically significant difference in broiler chicken meat, but only between the control and experimental groups with the highest addition of HS, at 450 ppm. Fletcher (1999) observed an increase in lightness in the color of chicken muscle tissue, and more so, when it also had a lower pH. This is in agreement with the other studies, where the effect of pH on the color of poultry meat was observed (Ozturk et al., 2012). It appears that HS may be associated with meat color, but the precise mechanism underlying this effect remains unclear (Bai et al., 2013). Darkening in muscles and an increase in their redness might indicate an increase in heme pigments because the red color in meat is mainly contributed to by a protein pigment called myoglobin and, to a lesser extent, hemoglobin (Yörük et al., 2004).

Specific parameters—dry matter, fat, water, proteins, water loss after cooking, phosphates, and pH of thigh meat—were all affected by the supplementation of HS in the chicken diet. Thigh meat obtained from broilers belonging to the experimental groups showed an increased content of phosphates and reduced pH (Table 3). The ability of HS to decrease pH in produced breast and thigh meat confers an advantage during storage, because fresh poultry products are usually kept refrigerated (Aksu et al. 2005). HS have a very good buffering capacity, stabilizing the pH in the entire digestive system. As ion exchangers and chelating agents, they increase the use of nitrogen and phosphorus and also increase the resorption of iron and copper (Rath et al. 2006). Yang et al. (2011) found that supplementation of broilers diets with Cu, Fe, Zn, and Mn significantly affected the abovementioned parameters on the breast
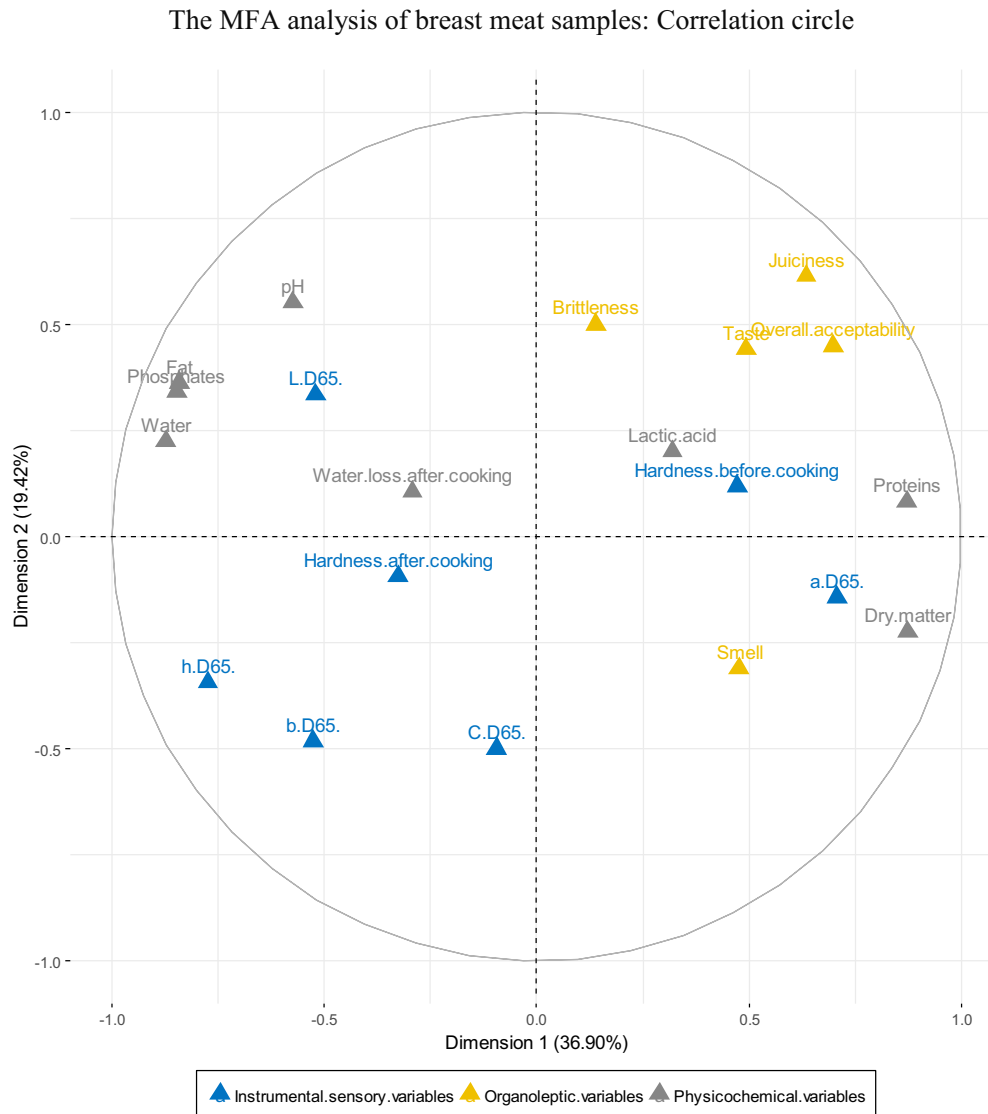
The MFA analysis of breast meat samples: Correlation circle

Figure 2. The MFA analysis of breast meat samples: correlation circle. Abbreviation: MFA, multiple factor analysis.

muscle of chickens. In this work, we confirmed that HS, in broilers fed a diet enriched with HS, affected meat color, which could also be a result of increased resorption of the minerals from the feed mixture.

In this study, we presented a relatively large amount of data and their subsequent evaluation through a commonly used ANOVA statistical method was considered as very similar. Because of that, MFA proved to be an applicable, fast, and suitable method for the purposes of studying the meat quality of broilers and for establishing the connection between physicochemical and sensory data, which were supplemented by instrumental measurements of meat texture and color. MFA is an analysis applied to tables in which a set of individuals is described by several groups of both categorical and quantitative variables (Escofier and Pagès, 2008).

One of the aims of MFA is to balance the influence of the groups of variables in the analysis in such a way that no single group (with many correlated variables for instance) dominates the first dimension of variability.

To do so, for each group of variables, a principal component method is performed (principal component analysis for a continuous group or multiple correspondence analysis for a categorical one) and then each value in the group is divided by the square root of the first eigenvalue (Josse and Husson, 2016).

MFA method was applied to the data of physicochemical and sensory evaluation supplemented with instrumental colorimetric and textural analysis, whereas results of breast and thigh meat samples were analyzed separately. The analysis extracted the most significant variables with a minimum loss of information. Kaiser's criterion (eigenvalue > 1) was applied to determine the number of final factors from the initial ones (Chapman et al., 2001).

The results of MFA showed that in breast meat samples, 3 selected components that explain more than 71% of the total variation in the data set. The first dimension (Dim1_a) explains 36.90% of variation, dimension 2 (Dim2_a) 19.44%, and dimension 3 (Dim3_a) 14.92% (Figure 1).

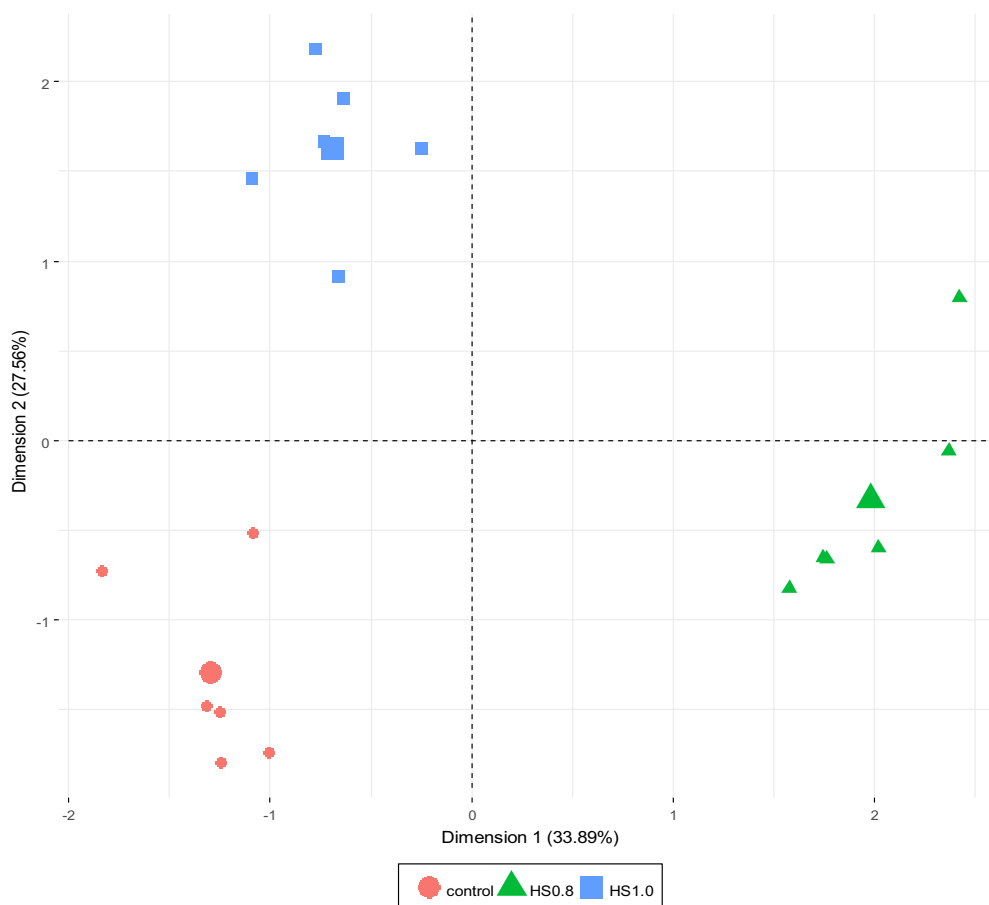The results of MFA analysis of thigh meat samples: Individuals

Figure 3. The results of MFA analysis of thigh meat samples: Individuals. Control, broilers fed with complete feed mixture; HS0.8, experimental group of broilers fed with diet enriched with 0.8% addition of humic substances; HS1.0, experimental group of broilers fed with diet enriched with 1.0% addition of humic substances. Abbreviation: MFA, multiple factor analysis.

Contribution of the analyzed data in $Dim1_a$ related to the sample variations (30.65%, $r = 0.92$), physicochemical (29.18%, $r = 0.90$), instrumental color and textural (21.76%, $r = 0.81$), and organoleptic variables (18.40%, $r = 0.73$). The highest contribution in $Dim1_a$ included the following variables: dry matter ($r = 0.87$), proteins ($r = 0.87$), a^* ($r = 0.71$), overall acceptability ($r = 0.70$), juiciness ($r = 0.63$), taste ($r = 0.50$), hardness before cooking ($r = 0.47$), smell ($r = 0.48$), L^* ($r = -0.52$), b^* ($r = -0.53$), pH ($r = -0.57$), h^* ($r = -0.78$), fat ($r = -0.84$), phosphates ($r = -0.85$), and water ($r = -0.90$), which correlated at a level $P < 0.05$. $Dim2_a$ was characterized by the contribution of the sample variations (46.67%, $r = 0.82$), organoleptic (28.35%, $r = 0.70$), and instrumental organoleptic properties (15.69%, $r = 0.65$) with physicochemical variables (9.79%, $r = 0.47$). In $Dim2_a$, the following variables correlated: juiciness ($r = 0.61$), pH ($r = 0.55$), brittleness ($r = 0.50$), b^* ($r = -0,48$), and C^* ($r = -0.50$). The first 2 dimensions explained a total of 56.34% of variance (Figure 2). Instrumental color and textural variables contribute especially to $Dim3_a$, with 40.62% ($r = 0.74$), followed by the sample variations 34.38% ($r = 0.62$), organoleptic 15.01% ($r = 0.50$), and

physicochemical variables 9.45% ($r = 0.35$). In $Dim3_a$, the following variables correlated at a level ($P < 0.05$): C^* ($r = 0.72$), b^* ($r = 0.63$), L^* ($r = 0.57$), and taste ($r = -0.57$).

Supplementation of HS in the chicken diet showed a decreasing trend of pH in the breast meat samples ($P < 0.001$). The pH decreased with increased concentration of HS (HS1.0). HS demonstrated the ability to decrease pH of the produced meat of the breast and thigh muscles, which can confer a great advantage during meat storage, during which time the meat is easier to sustain in pertinence to quality. Our findings about the effect of HS on decreasing pH trend are also confirmed by the results of Aksu et al. (2005) and partially by Disetlhe et al. (2019), which also indicate a decrease in pH after feeding with HS.

According to Kaiser's criterion, MFA analysis showed in thigh meat samples 2 selected components that explain more than 61% of the total variation in the data set. The first dimension ($Dim1_b$) explains 33.89% of variation, whereas dimension 2 ($Dim2_b$) explains 27.56% of variation (Figure 3).

The contribution of the analyzed data in $Dim1_b$ is related to the sample variations (45.76%, $r = 0.98$),

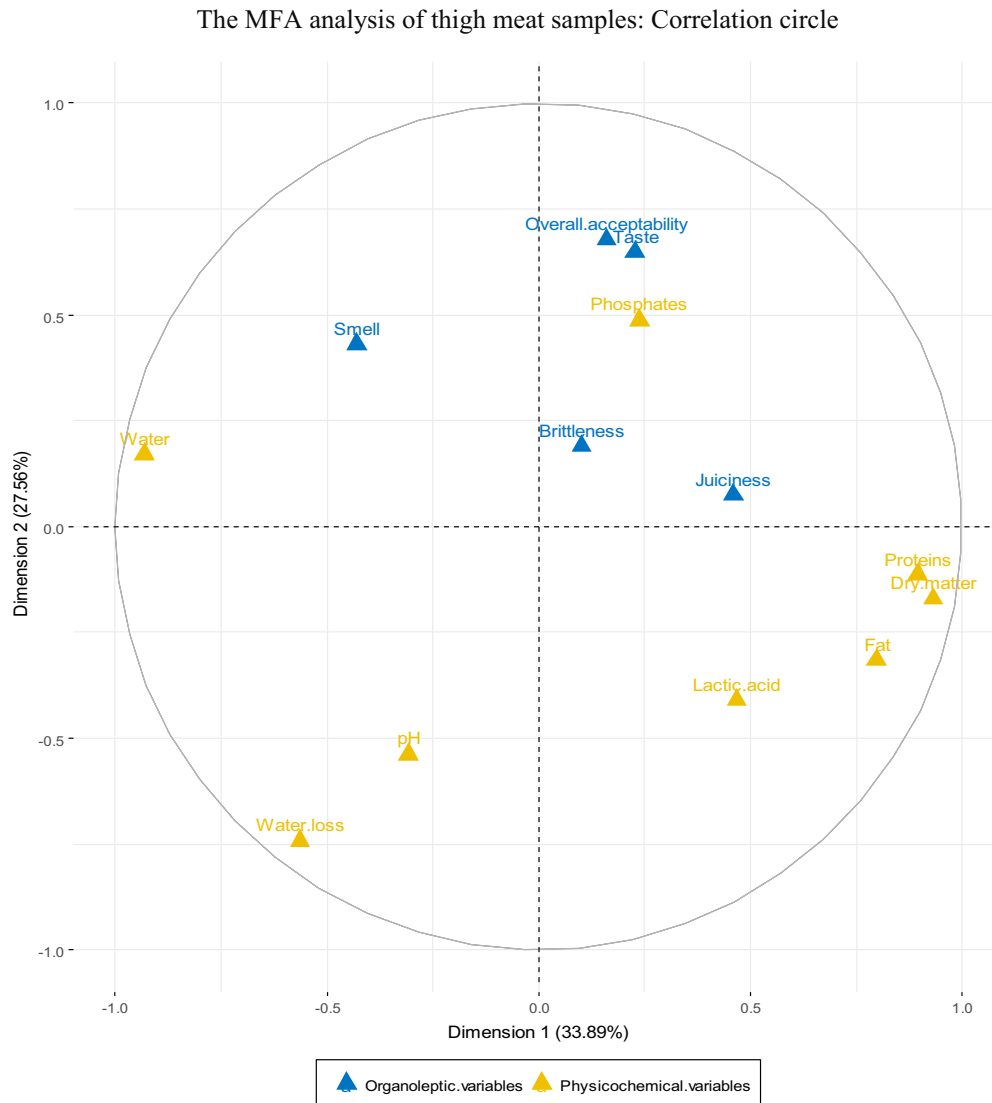The MFA analysis of thigh meat samples: Correlation circle

Figure 4. The MFA analysis of thigh meat samples: correlation circle. Abbreviation: MFA, multiple factor analysis.

physicochemical (43.61%, $r = 0.96$), and organoleptic variables (10.59%, $r = 0.52$). In Dim1_b, the following variables correlated at the level of significance $P < 0.05$: dry matter ($r = 0.93$), proteins ($r = 0.90$), fat ($r = 0.80$), water loss ($r = -0.56$), and water ($r = -0.93$). Dim2 was characterized by the contribution of the sample variations (50.39%, $r = 0.93$), organoleptic (29.93%, $r = 0.80$), and physicochemical variables (19.67%, $r = 0.90$). In Dim2_b, the following variables correlated: organoleptic overall acceptability ($r = 0.68$), taste ($r = 0.65$), phosphates ($r = 0.49$), pH ($r = -0.54$), and water loss ($r = -0.74$) (Figure 4).

The optimal sensory quality for broiler meat is usually reflected in meat color, tenderness, and water-holding capacity (Yang et al., 2011). The visual appearance of meat is one of the most vital meat quality attributes that influence visual acceptance of meat and meat products and subsequently, purchasing decisions by consumers (Disetlhe et al., 2019). Although the precise underlying mechanism of the effect of HS is unknown, it appears that HS contain minute quantities of several

minerals, including Fe, Mn, and Cu, which may influence meat color (Ji et al., 2006) and subsequently the consumers choice through the visual acceptance of the product.

MFA method used in this work was shown as a very useful and effective statistical tool for the assessment of physicochemical and organoleptic quality of breast and thigh meat samples of broilers fed a diet supplemented with HS. The results of correlations from MFA analysis between observed variables will be useful and could be used in further studies of the effects of HS in animal nutrition.

CONCLUSION

The supplementation of HS in the diet of broilers influenced the physicochemical and organoleptic variables of meat. We observed significant changes in dry matter, fat, water, proteins, phosphates content, and pH in both breast and thigh meat samples. The changes in phosphates content and pH was slightly lower in thigh

meat than in breast meat samples. Significantly lower results of water loss after cooking were observed in thigh meat samples obtained from broilers fed a diet with 1% concentration of HS. The smell of chicken breast meat after cooking was evaluated by a sensory panel with a statistically significant higher point score given to the experimental group. The color of breast meat samples changed in the variables of lightness and redness with a higher percentage of supplementation with HS. MFA method was, in the presented study, a useful and effective statistical tool for the assessment of physicochemical variables and organoleptic properties, which were supplemented with instrumental measurements of breast and thigh meat samples. The effect of 0.8 and 1.0% HS supplementation in broilers diet in 2 different meat samples (breast and thigh meat) were set as the main factors. The presented statistical tool showed the relations and correlations in variables of each sample group. Implementation of HS supplementation in the diet of broilers could be a beneficial method for their management.

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