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Full Length Research Paper

The effect of different probiotics on broiler meat quality

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Probiotics are the possibility to chouse growth stimulation by using physiological potential of animals. The aim of this study was to evaluate the use of different probiotics on poultry meat. The experiment was started by fattening 700 one-day-old chicks (5 groups), provenance Arbor Acres, both sexes, initial mass 40.07 ± 0.33 g. Fattening period was 42 days. Control group was fed with complete feeding mixture of standard raw material and chemical composition without probiotics and the same feed was given to other 4 groups (experimental groups) but with addition of different probiotics. Chemical parameters, pH and sensory analysis were determined according to ISO standards, color and texture were determined instrumentally. During examination the meat quality of drumstick meat in all five groups, we found that there was statistically very significant difference ($P < 0.01$), though not all. Application of probiotics in feed increased meat quality which is in relation to chemical composition and pH value, color, tenderness and sensory analysis of drumstick meat.

Key words: Probiotics, meat quality, sensory analysis, color, texture.

INTRODUCTION

Poultry meat production is paid more and more attention because of it's composition, poultry meat is particularly high in quantities of valuable protein, essential amino acids, fat, essential fatty acids, vitamins and minerals, makes high quality concentrated food and therefore plays an important role in human nutrition (Ivanovic, 2003; Givens, 2005). Not less important are sensory properties observed by consumers as color, texture, juiciness and flavor. One important factor that determine the quality of meat is the pH value.

For chicken meat, characteristic pH for drumsticks after slaughter is 6.54 (Liu and Niu, 2008). The second most important meat attribute is color that is caused by concentration of myoglobin, its chemical status on the surface of meat, structure and physical status of muscle proteins and the proportion of muscular fat. Color of meat depends also of the age, condition, diet and pH values (USDA, 2008).

Next meat characteristic that attracts consumers is tenderness and it depends on the age, species, sex, race and diet (Schreurs, 1999).

Meat flavor depends primarily on animal species, age, gender, raising and diet as well as of *post mortem* changes. One of the biggest challenges of the poultry industry is facing developing countries to work on improving production efficiency. The main goal was to increase the utilization efficiency of feed, which included the introduction of antimicrobials and other natural products with feed to body (Paryad and Mahmoudi. 2008).

Such natural products include probiotics. Current

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definition states that probiotics are supplements of living microorganisms in to food, causing effects in animal hosts by maintaining eubioze, which antibiotics are exluded from this term. In recent years, usually is uses the term direct-fed microbials (DFM) which implies the source of live microorganisms, including bacteria, fungi and yeasts. The use of probiotics achieved similar effects as antibiotics, but the difference is that the undesirable effects are avoided (residues, carence, resistance, allergies, genotoxicity etc.) (Sinovec et al., 2000). The mechanism of action of probiotics is not clearly defined. Some authors believed that probiotics act like a normal gut microflora, in one or more of the following ways: neutralizing toxins, microbial growth suppression, competition for places adhesive, causing disorder of metabolism of other bacteria or stimulation of immunity. But from the abovementioned, we must not disregard vitamin production, nor restoration of normal intestinal microflora after antibiotic therapy.

Economy, that is, the increase of productivity is primarily based on the increased digestibility and absorption of fat, proteins and carbohydrates**.** The aim of this study was to evaluate the usage of different probiotics on drumstick meat (water, fat, protein, ash, ph, color texture and sensory analysis).

MATERIALS AND METHODS

The experiment was started by fattening 700 one-day-old chicks, provenance Arbor Acres, both sexes, initial mass 40.07 ± 0.33 g. A total of 700 one-day-old Arbor Acres broilers were randomly allocated into 5 groups (140 chickens per group). The length of fattening the chicks was 42 days. Chickens were raised in an object without windows and with controlled microclimatic conditions. The heating was provided by equally distributed warm air, with controlled relative humidity 60- 70% during the first days of life and later 50- 60%. Ventilation is regulated automatically by securing the 0.8 m^3/min (cubic feed/ minutes/ bird) to the mat we use chopped straw length 8 to 10 cm. The chopped straw, length 8 to 10 cm, was used for litter. The thickness of the litter was 10 to 12 cm. The building was divided into 5 sections by plastic mesh. The density of population was 12 birds/m^2 of the floor (maximum stocking density of 30 kg/m at slaughter). Water has been provided from the nipple drinkers and there was 8 birds per nipple. Ambient conditions were in accordance with technological norms for this provenance, the Animal Welfare Act (2009) and Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 1999) were applied.

Diet of poultry

Nevertheless, the way of feeding and watering of Control group and experimental groups were identical. We fed the chickens with complete feeding mixture of standard raw material and chemical composition. A diet is shown in Table 1. The vitamin-mineral supplement was made according to the needs of the hybrids and did not contain coccidiostatics. Growth promoters were not added. Chickens were fed ad libitum with starchy food. Control group was fed with complete feeding mixture without probiotics.

First group- received in feed probiotic in the amount of 0.10% of the following composition: *Lactobacillus plantarum*, *Streptococcus* CFU/g); second group- received in feed probiotic in the amount of 0.05% of the following composition: *Streptococcus faecium cernelle* cernelle strain 68 (70 x 10^6 CFU/g); third group- received in feed probiotic in the amount of 0.01% of the following composition: *Bacillus cereus* IP 5832 (10^{10} CFU/g); fourth group- received in feed probiotic in the amount of 0.05% of the following composition: *Bacillus* CH 200 (1.6 × 10^9 CFU/g) and *Bacillus* CH 201 (1.6 × 10^9 CFU/g). Table 1

All birds were slaughtered and processed at 42 days of age. On the day of processing, all birds were transported in cages to the slaughterhouse. Slaughterhouse for poultry was equipped with lines and equipment for the slaughter. Slaughtering of poultry was started by hanging on the line and removal them by conveyor to the pool to stunning. Poultry was stunned with electricity voltage 49V, with the stem of each individual sinks into the water pool (the current flows through the water). Bleeding is performed automatically by cutting the blood vessels in the neck (vein jugulares) and were allowed to bleed for 120 s. After bleeding, birds were scalded at 54℃ for 150 s, followed by mech anical carcass defeathering, and the process was completed by removing the heads. In the evisceration room, the vent was cut with a plunging knife and an eviscerator pulled the viscera from the cavity. The slaughter process was completed with several internal and external washings of the carcasses. At the end, carcasses were cooled by a water-air method.

When examining the quality of drumsticks samples, we have carried out: chemical testing (determining the content of: moisture, ash, fat, proteins, pH value), testing of color and texture (tenderness) of meat instrumentally and with sensor analysis we have determined the difference in acceptability of drumstick samples.

Chemical composition and pH value

Moisture content was determined by ISO 1442 (1998), fat content by ISO 1443 (1992) and ash content by ISO 936 (1999). Protein content was calculated from nitrogen content multiplied with 6.25 using ISO 937 1992) and pH value by ISO 2917 (2004). Chemical parameters and pH were measured in meat 5 hours after slaughter.

Colour measurements

The color was measured on the fresh drumsticks meat of each poultry carcass (n = 100, in duplicate for each sample). CIE $L^*a^*b^*$ and CIEYxy color coordinates were determined using chromameter (Minolta Chromameter CR-400, Minolta Co., Ltd., Osaka, Japan) in D-65 lighting, with standard angle of 2° of shelter and 8 mm aperture of the measuring head. Results were expressed in CIE system, as the average values: y (average reflectance or brilliance, %), λ (dominant wavelength, nm) and P (color purity, %). In CIEL*a*b* results were given as the mean values: L* (psychometer light), a* (psychometer tone) and b* (psychometer chroma).

Determining the texture

Determining the meat texture was performed on a universal apparatus Instron (model 4301-5KN) with the use of contact extension according to Warner-Bratzler (Warner-Bratzler meat shear) in the following work conditions: applied force 250 N, speed 50 mm/ min, diameter 2.54 cm.

Sensory analysis

Selected (trained) evaluators participated in sensory analysis of

Table 1. Ingredients and analysed composition of feed mixtures.

Item	Starter 1-14 d	Grower 15-35 d	Finisher 36-42 d	Item	Starter 1-14 d	Grower 15-35 d	Finisher 36-42 d	Item	Starter 1-14 d	Grower 15-35 d	Finisher 36-42 d
	Ingredients, %				Ingredients, %				Analysed nutrient contents		
Grinded corn	53.50	62.00	64.00	Vitamin-mineral premix[1]	0.50	0.50	0.50	Dry matter, %	89.28	89.21	89.15
Grinded sunflower	5.00	5.00	5.00	Yeasts	2.00	1.00	1.00	Crude ash, %	5.79	5.96	5.44
Extracted grinded soybean	26.00	18.50	18.00	Corn gluten	2.50	4.40	4.20	Crude protein, %	22.23	21.34	19.48
Fish meal	4.00	2.00	-	Methionine	0.20	0.15	0.15	Crude fat, %	7.76	7.28	8.16
Calcium carbonate	0.80	0.80	0.80	Lysine	-	0.15	0.15	Crude cellulose, %	4.37	4.51	4.37
Dicalcium phosphate	1.30	1.70	1.90					Nitrogen-free extracts, %	49.12	50.12	51.69
Soybean oil	4.00	3.50	4.00					Ca, %	0.97	0.99	0.81
Sodium chloride	0.20	0.30	0.30					P, %	0.85	0.85	0.71
								ME, kcal/kg	3,165	3,138	3,213

[1]Vitamin-mineral premix contained per kg: period 1 to 35 d: IU: vit. A 1,500,000, vit. D_3 250,000; mg: vit. E 3,000, vit. K_3 300, vit. B_1 250, vit. B_2 800, vit. B_3 3,000, vit. B_6 350, vit. B_{12} 2, vit. C 2,000, vit. H 10, Ca-pantothenate 1,500, folic acid 100, choline 55,000, Mn 8,000, Fe 4,000, Co 40, Cu 800, Zn 5,000, Se 15, I 110, antioxidant 100; period 36- 42 d: IU: vit. A 1,500,000, vit. D_3 250,000; mg: vit. E 3,000, vit. K_3 300, vit. B_1 250, vit. B_2 800, vit. B_3 3,000, vit. B_6 350, vit. B_{12} 2, vit. C 2,000, vit. H 10, Ca-pantothenate 1,500, folic acid 100, choline 55,000, Mn 8,000, Fe 3,500, Co 40, Cu 800, Zn 5,000, Se 15, I 100, antioxidant 10,000.

drumsticks. We performed the selection of evaluators according to the ISO standard (ISO 8586:1993), and 20 evaluators participated in the evaluating process. The samples were prepared prior to the testing in an identical way. The skin from chicken drumsticks have been removed before thermal treatment by heating them on electric grill for about 20 min, until reaching the temperature of 80℃ in meat. After thermal treatment the samples were presented to evaluators on identical plastic plates which were marked. Their task was to rate the samples after testing the taste and smell so that the first place belongs to a sample that is, in their opinion, the most acceptable, 2nd to less acceptable, etc., and last place would belong to least acceptable sample (ISO 6564:1985). The evaluators were devided in 2 groups. Each group worked in 5 sessions, which means that 10 groups of samples were analyzed in 1 session. After 5 groups of samples, evaluators had 45 minutes to rest. Based on the number of compared samples, number of rankings and the difference between the sums of ranks in some of samples from the table, statistical significance of differences in acceptability is calculated in the level of $P < 0.05$ that is, $P < 0.01$ (ISO 8587:2006).

Statistical analysis

Basic parameters of the descriptive statistics included calculations of the arithmetic mean values, variability parameters of the investigated properties included

Table 2. Chemical composition, pH value of drumsticks samples.

Group	n	M ± SD				n	pH, five hours after slaughter
		Moisture (%)	Fat (%)	Protein (%)	Ash (%)		
Control	20	66.22±1.31^{a}	13.67±1.55^{a}	19.43±0.95^{a}	0.90±0.05	100	5.65±0.02^{a,b}
1	20	71.15±0.15^{b,h}	8.68±0.61^{b,h}	19.37±0.87^{a}	0.88±0.04^{a}	100	5.56±0.02^{a}
2	20	71.98±1.54^{b,h}	8.88±2.08^{b,h}	18.24±1.47^{b}	0.90±0.04	100	5.58±0.01^{a}
3	20	70.54±0.87^{c}	9.59±1.18^{c}	18.95±0.92^{b}	0.92±0.06^{a}	100	5.67±0.01^{a}
4	20	70.82±0.89^{c}	9.78±1.36^{c}	18.51±1.48^{h}	0.89±0.05	100	5.64±0.06^{a,c}

^{ns}-no statistically significant difference; $^{a-c}$Means within the same column with different superscripts differ significantly (P<0.01); ^{h}Means within the same column with different superscripts differ significantly (P<0.05).

determinations of standard deviations (SD) expressed in percents. Data obtained in investigations were analyzed by descriptive and analytical statistics, using SPSS-Excel (SPSS-Excel, 2002). The differences between 2 averages were compared by t-test at the level of significance of 99 and 95% (Hadzivukovic, 1991).

RESULTS

Examining the chemical composition of drumsticks (red meat), we found that the average water content was the lowest in control group (66.22 ± 1.31%), and the highest average water content was found in group 2 (71.98 ± 1.54%). Statistically highly significant difference (P < 0.01) in average water content was between group 2 and control group, group 2 and 3, group 2 and 4, group 1 and control group, group 1 and 3, group 4 and control group, group 3 and control group. Statistically significant difference (P < 0.05) was between group 2 and 1. In other groups that were compared (group 1 and 4, group 4 and 3) there was no statistically significant difference (P > 0.05) (Table 2).

The lowest average fat content in drumsticks was in group 1 (8.68 ± 0.61%), and the highest average fat content was in control group (13.67 ± 1.55%). There was a statistically highly significant difference (P < 0.01) in average fat content between control group and group 1, control group and group 2, control group and group 3, control group and group 4, group 4 and group 1, group 3 and group 1 group. In the other groups which were compared there was no statistically significant difference (P > 0.05) (Table 2).

The average protein content in drumsticks was the lowest in group 2 (18.24 ± 1.47%), and the highest average protein content was in control group (19.43 ± 0.95%). There was a statistically highly significant difference (P < 0.01) in average protein content between control group and 2, group 1 and 2, whereas statistically significant difference (P < 0.05) was between control group and 4, group 1 and 4. In the other groups that were compared there was no statistically significant difference (P > 0.05) (Table 2). In the tested drumsticks the average ash content was the lowest in group 1 (0.88 ± 0.04%), and the highest average ash content was in group 3 (0.92 ± 0.06%). Between group 3 and 1 there was a statistically significant difference (P < 0.05), in the other groups which were compared there was no statistically significant difference (P > 0.05) (Table 2).

The lowest average pH value was in group 1 (5.56 ± 0.02) and the highest average pH value was in group 3 (5.67 ± 0.01). Among the groups there was a statistically highly significant difference (P < 0.01) except control group and group 4 where was no statistically significant difference (P > 0.05) (Table 2).

During further research, we examined the effect of probiotics on the color, texture and sensory properties of drumsticks. Analyzing our results, that is, the mean of read and computer-calculated characteristics of color drumsticks samples on Minolta Chromameter and expressing in 2 systems (CIE, CIEL*a*b*), we can conclude, so to speak the "brightest" drumsticks were from group 3, and the "darkest" drumsticks were from group 4. The values we obtained for L*, a* and b* were from 50.32 (group 3) to 43.47 (group 4), from 6.77 (group 2) to 1.49 (control group) and from 15.96 (group 1) to 10.24 (group 3). For color parameters (y, L*, a*, b*) between all the groups that were examined, there was a statistically highly significant difference (P < 0.01) and for the color parameters λ i P between groups were statistically highly significant difference (P < 0.01) except group 3 and control group where was no statistically significant difference (P > 0.05) (Table 3). The results that we obtained indicate that individual samples differ only in nuance of color, but, visually are all acceptable.

Measuring the texture of drumsticks at the same given conditions we found that the "softest" was from group 3 (0.0240 ± 0.0002) (Table 4). Also, drumsticks in group 3 had the highest acceptability (Table 5). "Hardest" objective measurement of samples softness, between control group and groups that ate feed with probiotics drumsticks were found in control group (0.0400 ± 0.0014) (Table 4). According to the results established in there were a statistically highly significant difference (P < 0.01) (Table 4).

The results of sensory analysis are shown in Table 5. The obtained results showed that samples from control group and from group 4 were statistically significantly

Table 3. Characteristics of colour quality and texture of drumstick samples (CIE and CIEL*a*b* system)

Group	n	CIE system M ± SD			CIEL*a*b* system M ± SD		
		y (%)	λ (nm)	P (%)	L*	a*	b*
Control	100	18.52±0.08	580.00±0.50	12.22±0.03	50.12±0.08	1.49±0.04	10.76±0.04
1	100	13.61±0.05	582.00±0.40	26.62±0.05	43.56±0.04	4.57±0.04	15.96±0.05
2	100	14.71±0.06	586.50±0.30	21.30±0.04	45.23±0.06	6.77±0.04	13.47±0.05
3	100	18.69±0.09	580.00±0.50	12.22±0.05	50.32±0.09	2.47±0.05	10.24±0.07
4	100	13.48±0.06	586.00±0.50	23.66±0.05	43.47±0.05	5.56±0.04	15.13±0.05

Table 4. Characteristics texture of drumstick samples.

Group	Texture of drumsticks	
	n	M ± SD
Control	100	0.0400±0.0014
1	100	0.0281±0.0002
2	100	0.0330±0.0001
3	100	0.0240±0.0002
4	100	0.0380±0.0003

Table 5. Differences in the acceptability of drumsticks.

Group		Acceptability of drumsticks				
		n	M ± SD		Sx	CV
Mark of product		Control	1	2	3	4
The sum of the ranks		418	230	286	215	351
Difference according	Control	-	188^{a}	132^{a}	335^{a}	67^{h}
	1	-	-	56^{ns}	15^{ns}	121^{a}
	2	-	-	-	71^{h}	65^{h}
	3	-	-	-	-	136^{a}

^{ns}-no statistically significant difference; ^{a}Means within the same column with different superscripts differ significantly (P<0.01); ^{h}Means within the same column with different superscripts differ significantly (P<0.05)

less acceptable (P < 0.01) compared to the samples from group 1, group 2 and group 3.

DISCUSSION

Water, proteins and fat are major constituents of the meat and their qualitative and quantitative relationship determines the quality, in the other words, nutritional value of meat.

Our results with respect to protein, fat and water are in agreement with results Sazedul et al. (2010), where authors added different doses of probiotics to feed of broilers during fattening (from d 1 up to 8 wk when they were slaughtered). By analyzing the chemical composition of drumsticks they found that the total protein content was statistically significantly higher (P < 0.05) after the addition of probiotics (23.89 ± 0.27), comparated to control group (21.94 ± 0.04), total fat content (0.73 ± 0.10) was statistically significantly different (P < 0.05) lower in drumsticks originated from chickens that were fed probiotics in feed compared to control group (1.04 ± 0.11). The percentage of water in drumsticks (73.84 ± 0.41) that were given a probiotic in feed was also a significantly different (P < 0.05) lower compared to control group (74.00 ± 0.61). Total ash content gave no statistically significant difference (P > 0.05) between all five groups.

Paryad and Mahmoudi (2008) examined the effect of yeast on dry matter, crude protein and ether extract in

chicken meat. In the experiment they had a control group and 2 groups which received different amounts of yeast (0.5 and 1.5%). Yeast was added to the usual composition of feed and fed the chickens during fattening (from d 1 to 42). They found that drumsticks of chicks that received rations which contained 1.5 and 2% *S. cerevisiae* had got higher ($P < 0.05$) dry matter, crude protein and ether extract percentage.

Meanwhile, drumsticks of chicks which received rations containing 2% *S. cerevisiae* had got higher ($P < 0.05$) ether extract percentage compared with control, 0.5 and 1.5% *S. cerevisiae.* The results of the above mentioned authors refer to those proteins are not consistent with the results we obtained in this work. The results of the abovementioned authors refer to those moisture are consistent with the results we obtained in this work.

Our results, obtained in this study of impact probiotics on the percentage of fat in drumsticks of chickens that received probiotics in feed, are in accordance with the results obtained by Ignatova et al. (2009). These authors examined the effect of probiotics on total protein, fat and ash. In the experiment, they used probiotics which contained bacterial species. They added them to the feed wherewith was chickens from experimental group fed during entire fattening. They determined that the probiotic decreased fat in drumsticks of experimental group compared to control group. Karaoçlu et al. (2004) in their experiment had 3 groups of chickens. One was the control group and in other 2 groups, probiotics were added to feed during entire fattening period. They examined effect of probiotics on pH value of skin and pH value of drumsticks. They found that pH value in drumsticks of control group was 6.07a ± 0.20, in experimental group 1 was 6.02b ± 0.18, in experimental group 2 was 6.09a ± 0.19. pH value from experimental group 1 was a statistically significantly lower ($P < 0.05$) compared to the other 2 groups. Our results obtained during the research and which refer to pH value of drumsticks from experimental groups compared to control group are partially in agreement with the results of abovementioned authors. But, our results are consistent with the results of Aksu et al. (2005) who examined the effect of probiotics in broiler feeding by evaluating meat quality of poultry carcasses. Feeds supplemented with different levels of probiotics (0.0%, 0.1 and 0.2%) containing *Saccharomyces cerevisiae* were used in period for 49 d. The drumsticks were analyzed for pH. In this experiment, the probiotic in broiler diets increased pH values of drumstick ($P < 0.01$).

One of the most important characteristics of food quality in a broad sense, including chicken meat, is color. Color can be defined as a combination of visually perceived information contained in the light which is emitted or scattered by sample. Relatively small changes in light can produce major changes of color rather than long range of concentrations of pigments. Our results are in agreement with results of Karaoçlu et al. (2004). Researchers in their experiment with pH values, examined the effect of probiotics on the color of skin and drumsticks. They concluted that use of probiotic affected L^* and b^* values in skin and drumsticks ($P < 0.05$). Probiotic groups (group 1 and group 2) had lower values than control group ($P < 0.05$) Whereas L* and b* values in drumsticks skin were higher, a^* value was higher in drumstick meat.

Pelicia et al. (2004), examined the effect of different probiotics, prebiotics and yeast to the texture of meat drumsticks. Neither statistically significant difference ($P > 0.05$) was found between the groups in the experiment that received food supplements and the control group, nor in the second experiment (Pelicia et al., 2004a) when biological and chemical growth promoters were added in feed for chickens. The results that we obtained with instrumental measurements of hardness of meat do not comply with the abovementioned author.

The sensory properties of meat can be influenced by different components when added to feed for chicks. Pelicia et al. (2004a) formed in the experiment 4 groups of chickens and fed them during their fattening by adding biological and chemical growth promoters with coccidiosis vaccine and anti-coccidiosis. The sensory analysis determined that drumstick of chickens that were fed with the biological growth promoters with anti-coccidiosis is statistically significantly more acceptable ($P < 0.05$) in relation to drumstick of chickens fed with chemical growth promoters with anti-coccidiosis. The taste of chicken drumstick that were fed with the biological growth promoters with anti-coccidiosis was statistically significantly more distinct ($P < 0.05$) in relation to drumstick of chickens fed with biological growth promoters with coccidiosis vaccine. In relation to the juiciness there was a statistically significantly difference ($P < 0.05$) between chicken drumstick which were fed with chemical growth promoters with coccidiosis vaccine compared to drumstick of chickens which were fed with the biological growth promoters (Table 4).

In other qualitative parameters, there was no statistically significant difference ($P > 0.05$). Our results could not be fully comparable with the findings of the above mentioned authors. But, if we look at effects of biological growth promoters on the sensory properties, then our results agree with them.

CONCLUSION AND APPLICATIONS

After all abovementioned, we might agree with the following:

(i) The presence of probiotics in broiler feeding significantly decreases the lipid component and increase water content in drumstick meat. This affect was

observed in a group of broilers who received the mix of *Bacillus licheniformis* and *Bacillus subtillus* through the feed;
(ii) Added probiotics in broiler feeding period do not affect on protein and ash content in drumstick meat;
(iii) According the mean values meat color characteristics of samples from all four systems were different – the most bright drumstick meat was from group four and the most dark drumstick meat was from group five;
Iv) Instrumental measurements of hardness of chicken meat gave us that the meat from group four was the softest and the meat from group one the hardest;
(v) Acceptance criteria of drumstick meat from all groups were under the criteria for control group, except for the group that was fed with addition of mixture containing *Bacillus licheniformis* and *Bacillus subtillus*.

At the end we might say that application of probiotics during fattening period increased meat quality in relation to chemical composition, pH, color, tenderness and sensory analysis of drumstick meat.

Agreement or disagreement of our results with results from all above mentioned authors was expected, because all of them used different combination of probiotics from ours. Therefore we could say that all probiotics influence on meat quality parameters.

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