African Journal of Agricultural Research Vol. 7(14), pp. 2191-2196, 12 April, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1693
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

Influence of probiotics on quality of chicken meat

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Accepted 6 March, 2012

One of the biggest challenge that faced chicken industry in the developing countries was the increasing production efficiency, including the increase of feed conversion and meat quality, which includes addition of antimicrobial agents and natural products. One of these natural products are probiotics. The aim of this study was to evaluate the act of different probiotics on pH, chemical composition of meat (water, proteins, lipids and ash), some fatty acids distribution, acid number and acidity in abdominal fat tissue. The experiment started by fattening 300 one day-old chicks, provenance Ross 500, of both sexes with an initial mass of 41.00 ± 0.30 g. The chicks were divided into three groups (100 chickens per group). Analyses were determined according to International Standardization for Organization (ISO) standards. During examination of the meat quality of drumstick and breast meat in all three groups, it was found that there was statistically significant difference. Application of probiotics in feed has influence on meat quality, which is in relation to the chemical composition and pH value of drumstick and breast meat, and some fatty acids distribution, acid number and acidity in abdominal fat tissue.

Key words: Probiotics, meat quality, fatty acids, acid number, acidity, abdominal fat tissue.

INTRODUCTION

Chicken meat has a significant role in human nutrition because it contains high quality proteins and essential amino acids, lipids and essential fatty acids, vitamins and minerals (Givens, 2005). Besides the expected quality, chicken meat production occupies more attention because it is quicker and cheaper than the production of other meats (pork, beef etc), there is no religious or cultural restrictions for consumption, has desirable sensory parameters, positive aspect on human health (chicken meat has low fat and high protein content) and an acceptable price (Ivanović, 2003).

One of the biggest challenge that facing chicken industry in the developing countries is increasing production efficiency, but, first of all, the increasing of feed conversion, which meant addition of antimicrobial agents and other natural products (Paryad et al., 2008). One of these natural products were probiotics. Novel definition says that probiotics are live organism nutrition supplements, that produce effects in animal hosts through maintaining of eubiosis, which exclude antibiotics.

Recently, DFM (direct-fed microbials) is used very often, which means the source of live micro-organisms including bacteria, fungi and yeasts (Sinovec, 1998). Probiotics represent the method of choice for growth stimulation using physiological potentials and mechanisms of the host animal. By using probiotics, similar effects as to using antibiotics are achieved but the only difference is that the undesirable effects are avoided (residues, waiting period, resistance, allergies and genotoxicity etc) (Sinovec et al., 2000).

Mechanisms in the act of probiotics has not been clearly defined. Some authors said that probiotics worked similarly to normal micro flora of digestive tract in one or many ways by neutralizing toxins, suppressing micro floral growth, forcing competition for adhesive sites, causing metabolic disorders in other bacteria or by stimulating immunity. Beside these, we must not disregard vitamin production or restoration of normal intestinal micro flora after antibiotic therapy (Fuller, 1989). Economy, that is, the increase of productivity is primarily

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Table 1. Ingredients and analyzed composition of feed mixtures.

Variable Feed (%)	Starter (1-14 day)	Grower (15-35 day)	Finisher (36-42 day)
Ground corn	48.82	58.20	64.10
Soybean meal	11.50	8.00	-
Soy grits	25.60	20.00	22.50
Sunflower meal	10.00	10.00	10.00
Methionine	0.13	0.05	0.10
Salt	0.30	0.30	0.30
Monocalcium phosphate	0.80	0.75	0.40
Calcium carbonate	1.65	1.50	1.40
Adsorbent	0.20	0.20	0.20
Vitamin-mineral premix[1]	1.00	1.00	1.00
Chemical composition			
Moisture (%)	11.15	11.20	11.20
Raw ash (%)	5.97	5.43	4.70
Raw protein (%)	22.31	19.39	17.35
Raw fat (%)	6.73	6.05	6.64
Raw cellulose (%)	5.09	5.01	4.93
Non-nitrogen containing (%)	48.75	52.92	55.18
Energy (kcal kg^{-1})	3.02	3.08	3.18
Lysine (%)	1.15	0.96	0.82
Methionine-cystine (%)	0.90	0.71	0.70
Calcium (%)	0.94	0.85	0.72
Phosphorus (%)	0.65	0.61	0.51

Vitamin-mineral premix contained per kg: [1]period 1- 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.

based on the increased digestibility and absorption of lipids, proteins and carbohydrates. The aim of this study was to evaluate the act of different probiotics on the chemical composition of meat (water, proteins, lipids, and ash), the some fatty acids distribution, acid number and acidity in abdominal fat tissue.

MATERIALS AND METHODS

The experiment started by fattening 300 one day-old chicks, provenance Ross 500, of both sexes, with an initial mass of 41.00 ± 0.30 g. The chicks were divided into three groups (100 chickens per group). Experiment was conducted in accordance with the principles and guidelines for the care and exploitation of domestic animals in science (FASS, 1999), and with recomendations issued from International Institute of Life Sciences (Cromwell et al., 2003). Facility for chicken accomodation was in the farm and it was used for the experiment only. It was ecologicaly safe and disinfected. Chicks were vaccinated first day at the farm against Newcastle disease and infective bronchitis. Ambient conditions were in accordance with technological norms for this provenance and the Animal Welfare Act was applied.

The length of chicks fattening was 42 days. Chicks consumed water and feed *ad libitum* during the experiment. Upkeep, the way of feeding and watering of control and experimental groups were identical.

Diet of poultry

Table 1 gives details of the ingredients and analyzed composition of feed mixtures.

Probiotic composition

1. Group 1: They received probiotic through feed in amount of 0.05% of the following composition *Streptococcus faecium cernelle* 68 (70×10^6 CFU/g).
2. Group 2: They received probiotic through feed in amount of 0.01% of the following composition *Bacillus cereus* IP 5832 (10^{10} CFU /g)
3. Control group marked with C was fed with complete feeding mixture without probiotics.

After slaughtering, carcasses were cooled by a water-air method.

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

Group	n	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	n	pH
Control	25	68.62 ± 0.40^{x}	$9.77 \pm 0.30^{b,z}$	19.83 ± 0.57^{z}	1.30 ± 0.07^{x}	100	5.60 ± 0.02^{y}
1	25	71.48 ± 0.45^{z}	$8.38 \pm 0.33^{a,x}$	18.74 ± 0.40^{x}	1.30 ± 0.06^{x}	100	5.50 ± 0.03^{x}
2	25	71.04 ± 0.52^{y}	9.09 ± 0.27^{y}	18.45 ± 0.33^{y}	1.38 ± 0.06^{y}	100	5.62 ± 0.02^{z}

$^{a, b}$ Means within the same column with different superscripts differ significantly (P<0.05); $^{x, y, z}$ Means within the same column with different superscripts differ significantly (P < 0.01).

Samples for chemical analysis

For the investigation of chemical composition and pH value, we used chicken breast (muskulus pectoralis superficialis and musculus pectoralis profundus) and drumsticks (muskulus gastrocnemius, muskulus biceps femoris and muskulus peroneus longus).

The method employed in the analysis is as highlighted as follows:

1. Total fat content was determined by ISO 1443 (1992).
2. Moisture content by ISO 1442 (1998).
3. Protein content was determined by ISO 937: 1992.
4. Ash content by ISO 936 (1999).
5. pH value was determined by ISO 2917 (2004).
6. Acid number and acidity were determined by ISO 660 (1996).

Folch-Lees method was applied for the lipid extraction from the abdominal fat tissue. After the lipid hydrolysis, the esterification of fatty acids to methyl esters was performed. Fatty Acid Methyl Ester (FAMEs) analysis was performed by gas chromatography technique (GC6890N, Agilent Technologies, USA) with external standard method using a standard of FAMEs mix 37 ("Supelco", USA). Chemical parameters and pH were measured in meat 5 h after slaughtering.

Statistical analysis

Data obtained in the investigations performed in this study were analyzed by descriptive and analytical statistics, using SPSS-Excel (Microsoft Office XP, Microsoft Excel 2002 for Microsoft Windows, version 10). Basic parameters of the descriptive statistics were the arithmetic mean values (M) and standard deviations (SD). The differences between the averages were compared by t-test at the level of significance of 99 and 95% (Hadzivukovic, 1991).

RESULTS

Quality of drumstick meat

Chemical composition of chicken drumstick meat and pH values are shown in Table 2. From Table 2, we could see that the water content was the highest in group 1 (71.48 ± 0.45), and the lowest in the meat of chicken drumsticks from C group was (68.62 ± 0.40). Between compared groups was a significant statistical difference at (P < 0.01).

The smallest value of fat was presented in the drumstick meat from group 1 (8.38 ± 0.33), and the largest value from C group (9.77 ± 0.30). Between C group and group 2, there was a significant statistical (groups 2 and 1, C group and group 1), there was also a significant statistical difference at (P < 0.01).

The highest content of protein was in the drumstick meat from C group (19.83 ± 0.57), and the lowest was in the meat from group 1 (18.74 ± 0.40). Between all compared groups was a significant statistical difference at (P < 0.01).

Ash content in C group (1.30 ± 0.07%) and group 1 (1.30 ± 0.06 %) was almost the same. Between these compared groups, there was no significant statistical difference at (P > 0.05). Ash content in group 2 was 1.38 ± 0.06%. Between compared groups (groups 2 and C, groups 2 and 1), there was a significant statistical difference at (P < 0.01). pH was the lowest in the drumstick meat from group 1 (5.50 ± 0.03), and the highest in the meat from group 2 (5.62 ± 0.02). Between these compared groups was a significant statistical difference at (P < 0.01).

Quality of breast meat

Examining the chemical composition of chicken breast meat, we found that the water content average was the lowest in samples from the first group (71.95 ± 0.49), and the highest in samples from the second group (72.98 ± 0.25%) (Table 3). Results from the Table 3 showed that between the compared groups, there was a significant statistical difference at (P < 0.01).

The lowest average of fat content was found in the samples from the second group of chickens (2.33 ± 0.49%), and the highest average fat content was found in the samples from the control group of chickens (3.32 ± 0.53%). From Table 3, we can see that between group C and group 1, there was no significant statistical difference (P > 0.05), but between group C and group 2, and group 1 and group 2, there was a significant statistical difference at (P < 0.01).

The average protein content was lowest in the samples from the control group (23.38 ± 0.13%), whereas, it was the highest in the samples from the first group (23.91 ±0.37%). Between groups 1 and 2, and groups C and 2, there exists a significant statistical difference at (P <0.05), and between groups 1 and C, there was a significant statistical difference (P < 0.01) also.

The average content of ash was lowest in the samples from the second group at (1.06 ± 0.03%), and the

Table 3. Chemical composition, pH value of chicken breast meat.

Group	n	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	n	pH
Control	25	72.28±0.30^{y}	3.26±0.30^{z}	23.38±0.13^{a,x}	1.08±0.05^{ns}	100	5.72±0.01^{y}
1	25	71.95±0.49^{x}	3.06±0.52^{y}	23.91±0.37^{c,y}	1.07±0.02^{ns}	100	5.68±0.01^{x}
2	25	72.98±0.25^{z}	2.33±0.49^{x}	23.63±0.51^{b}	1.06±0.03^{ns}	100	5.74±0.01^{z}

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

Table 4. Content of oleic, lauric and ricinolic acid, acid number and acidity in abdominal fatty tissue (%).

Group	n	Oleic acid %	Lauric acid %	Ricinolic acid %	n	Acid number	Acidity
Control	10	0.45±0.02^{x}	0.29±0.03^{x}	0.46±0.03^{x}	25	0.89±0.02^{x}	1.50±0.03^{x}
1	10	0.54±0.03^{y}	0.34±0.03^{y}	0.55±0.02^{y}	25	1.03±0.03^{y}	1.79±0.03^{y}
2	10	0.58±0.03^{z}	0.38±0.02^{z}	0.60±0.02^{z}	25	1.20±0.02^{z}	1.97±0.02^{z}

$^{x, y, z}$ Means within the same column with different superscripts differ significantly ($P < 0.01$).

difference at ($P < 0.05$), and between compared groups highest was from the control group having a value of (1.09 ± 0.04%). Between these compared groups, there was no significant statistical difference at ($P<0.05$).

The lowest pH was measured 5 h after slaughtering in the chilled breast meat of the first group (5.68 ± 0.01), and the highest was in the second group (5.74 ± 0.01). Between all compared groups, there was a significant statistical difference at ($P < 0.01$).

Fatty acid composition

In Table 4, there are shown results for influence of probiotics on fatty acids from abdominal fatty tissue. The lowest content (%) of oleic acid was in abdominal fatty tissue from C group (0.45 ± 0.02) and the highest from group 2 (0.58 ± 0.03). Between all compared groups, there was a significant statistical difference at ($P < 0.01$).

The lowest content (%) of lauric acid was in abdominal fatty tissue from C group (0.29 ± 0.03) and the highest from group 2 (0.38 ± 0.02). Between all compared groups, there was a significant statistical difference at ($P < 0.01$).

The lowest content (%) of ricinolic acid was in the abdominal fatty tissue from C group (0.46 ± 0.03) and the highest from group 2 (0.60 ± 0.02). Between all compared groups, there was a significant statistical difference at ($P < 0.01$).

Table 4 showed the influence of probiotics on acid number and acidity in abdominal fatty tissue. The lowest acid value (0.89 ± 0.02) and acid level (1.50 ± 0.03) were in the abdominal fat tissue from C group, and the highest acid value (1.20 ± 0.02) and acid level (1.97 ± 0.02) were in the abdominal fat tissue from group 2. Between all compared groups, there was a significant statistical difference at ($P < 0.01$).

DISCUSSION

Drumstick meat

Our results showed that protein and water content in drumstick meat are not in accordance with the results obtained by Sazedul et al. (2010), but are in accordance for fat content. These authors in their experiment added different amounts of probiotics *Salicornia herbacia* (*L. acidophilus*, *L. plantarum*, *Bacillus subtilis* and *Saccharomyces cerevisiae*) in chicken feed during the growth period (from the first day till the eighth week when they were slaugtered). Analyzing chemical composition of drumstick meat, they found that protein content was statistically higher at ($p < 0.05$) in chicken meat after probiotics addition (23.89 ± 0.27) when compared to the control group (21.94 ± 0.04), total fat content (0.73 ± 0.10) was statistically lower ($p < 0.05$) in drumstick meat which were from chickens who had received probiotics as compared to the control group (1.04 ± 0.11).

From our results, water content was the lowest in drumstick meat from control group and they are not in accordance with the results obtained by Sazedul et al. (2010). The results were obtained from these authors for water content in drumstick meat who received probiotic (73.84 ± 0.41). Although feed was also statistically lower at $p < 0.05$ than in those obtained from the control group (74.00±0.61), there was no significant statistical difference ($P<0.05$) between the compared groups for total ash content. In our results for total ash content, there was also no significant statistical difference ($P<0.05$) between the compared groups. Our results obtained for water and fat content in drumstick meat are in accordance with the results obtained by Khaksefidi and Rahim (2005). These authors also in their experiments observed that there was a significant statistical difference in water and fat content in drumstick meat from groups

that received probiotics through feed as compared to the control group. Water content in drumstick meat from groups that received probiotic was 72.40, but drumstick meat from control group was 71.35. Fat content in drumstick meat from groups that received probiotic was 4.87, but drumstick meat from control group was 7.06. Our results for protein content were not in accordance with results obtained from aforementioned authors. In our results, the highest protein content was in the drumstick meat from control group. But from the experiments of the aforementioned authors, higher protein content was found in the drumstick meat from groups that received probiotic. Our results obtained for ash content were in accordance with results obtained from aforementioned authors. Results shown in Table 2 for fat content in drumstick meat revealed that the probiotic reduced fat content in the meat from group 1 was in accordance with the results obtained by Kalavathy et al. (2006). These authors reported that the fat contents of the muscle and carcass were significantly ($P < 0.05$) lower in the *Lactobacillus* cultures (LC) - fed broilers when compared to the control broilers. Our results were not in accordance with these results.

Our results obtained for measuring pH in drumstick meat five hours after slaughtering are partially in accordance with the results obtained by Aksu et al. (2005). These authors also in their experiment added probiotics (*S. cerevisiae*) through feed and investigated the influence on meat pH. Results from their investigation showed that probiotic increased pH in breast and drumstick meat ($P < 0.01$). In our investigation, probiotic added to group 1 decreased and probiotic added to group 2 increased the pH value when compared to the control group.

Breast meat

Our results (Table 3) are not in accordance with the results obtained by Hascik et al. (2009). In their experiment, they also added probiotic in their chicken feed. Their conclusion was that there was a difference in the content of dry matter and proteins when the compared the values obtained by chicken meat analysis from control group and meat from chickens that received probiotics in feed, but that the difference was not statistically significant.

Our results for water, total protein, total fat and ash content were in accordance with the results obtained by Ignatova et al. (2009). Authors added to chickens through the feed mix of probiotics (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus reuterii, Bifidobacterium bifidum, Bifidobacterium animalis Bifidobacterium infantis*). Their results for control group were: water 24.77 ± 1.77%, total protein 19.85 ± 0.81%, total fat 4.27 ±1.01%, total ash 1.01 ± 0.11% and for groups that received probiotic was: water 24.86 ± 1.06%, total protein 20.38 ± 0.61%, total fat 3.77 ± 0.98%, total ash 1.07 ± 0.05%. As seen from the results, probiotics had influence on meat quality.

In regard to pH, results obtained (Table 3) were in accordance with that obtained by Fatma (2010), by measuring pH 24 h after slaughter. Author cited that there was statistically a significant difference between pH of meat samples from chickens in the control group and meat samples of chickens that received probiotics in feed. On the other hand, our results were not in accordance with the results obtained by Pelicano et al. (2005). Authors measured pH 5 h after the slaughter and found no statistical significant difference between meat samples from chickens that did not receive probiotics.

Results shown in Tables 2 and 3 are in accordance with the results obtained by Ivanović et al. (2005). These authors investigated the influence of four different probiotics on acid value and acidity in fat tissue from hybrid Arbor acres. In this investigation, it was also confirmed that acid value and acidity were the lowest in the meat from control group. Between control group and other groups in the expereiment , there was a statistically significant difference at ($P < 0.01$).

Fatty acids

In the same experiment Ivanović et al. (2005) investigated on the content of oleic, lauric and ricinolic acid. The lowest average content for all three fatty acids was in the abdominal fatty tissue from control group chickens. Between control group and other groups in the expereiment, there was a statistically significant difference at ($P < 0.01$).

Our results for oleic acid content (Table 4) are also in accordance with the results obtained by Kalavathy et al. (2006). Supplementation of Lactobacillus in the broiler diets significantly ($P < 0.05$) reduced the oleic acid ($C_{18:1}$) levels of the liver, muscle and carcass. Conclusions of Kalavathy et al. (2006) were that cultures of Lactobacillus reduced the fat content in broiler chickens, but they have very little potential to modify the composition of fatty acids.

Conclusion

After all aforementioned, we might conclude on the following that:

1. The addition of probiotics in chicken feed significantly decreased fat and increased the water content in drumstick and breast meat.
2. The addition of probiotics in chicken feed caused big differences in acid value and acidity indicating that probiotics could have effect on fatty acid oxidation and change the taste of the meat.

3. The application of probiotics during fattening period increased meat quality in relation to the chemical composition of chicken meat.

The differences of our results with that from all aforementioned authors were expected because we all used different combination of probiotics in carrying out the experiments.

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