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INFLUENCE OF PROBIOTICS CLOSTAT® AND LAKTINA® ON THE QUALITY OF MEAT OF PHEASANTS

Tandzhu MEHMEDOV[1], Eva GYUROVA[2], Stanislav RADANSKI[1], Zapryanka SHINDARSKA[1]

[1]University of Forestry, Faculty of Veterinary Medicine, 10 Kliment Ohridski Blvd, 1756, Sofia, Bulgaria, Phone: +35928192934, Email: tanju_mm@yahoo.com

[2]National Diagnostic and Research Veterinary Medical Institute, National Centre of Food Safety , 15 Pencho Slaveikov Blvd,1606, Sofia, Bulgaria, Phone: +35929521277, Email: evaguirova@abv.bg

Corresponding author email:tanju_mm@yahoo.com

Abstract

Probiotics are widely accepted as an alternative to the nutritive antibiotics in poultry production as opposed to farm breeding pheasants.The aim of the study was to investigate the influence of probiotics CloSTAT® and Laktina® on meat quality of 90 day-old pheasants. The experiment was conducted with 90 newly hatched pheasants (Phasianus colchicus colchicus), divided into 3 groups of 30 birds in each group, floor breeding with free access to food and water for 90 days. The three groups were fed with a standard compound feed for pheasants ad libitum, for the experimental groups as follows:for the second group (group B) probiotic CloSTAT® (0,5 g/kg feed) was added; and for the third group (group C) probiotic Laktina® (0,5 g/l of drinking water) was added. After completion of the experiment from each group were slaughtered 5 pheasants for meat sampling of the breast and leg. The following indicators were analysed: pH 24h post mortem, the water holding capacity, colour of the meat, content of myoglobin, protein and ash content. The results of the experiment showed that the use of the probiotics CloSTAT® and Laktina® increases the pH of the breast muscle, lightens the colour and decreases the myoglobin content in the leg and breast muscle, increases the water and mineral content in the leg muscle. The probiotics impact the protein metabolism in leg and breast muscle in different ways. The use of probiotic Laktina ® leads to the accumulation of a larger amount of proteins in the breast muscle, and the use of CloSTAT® - reduces their amount in the leg muscle.

Keywords: *pheasants, meat quality, probiotics.*

INTRODUCTION

The widespread use of antibiotics in livestock farms to stimulate the growth, the increase of the efficiency of nutrition and the prevention of intestinal infections in recent years have led to the development of resistance in certain species of bacteria in the gastro-intestinal tract and the accumulation of residues in meat.

The use of probiotics as an alternative to the nutrition antibiotics is widespread in poultry, especially since some countries have banned certain antibiotics that are often included in the rations as growth promoters.

Probiotics are defined as viable microorganisms (bacteria or yeast) that competitively exclude colonization of intestinal pathogens and demonstrate a beneficial effect on the health of the host when ingested (Salminen et al., 1998).

Probiotics are widely used in poultry farming as opposed to farm bred pheasants. Many authors study the impact of probiotics on meat quality in broilers (Kabir, 2009; Ivanović et al., 2012; Hossain et al., 2012; Maiorano et al. 2012).

There are a number of studies on yield, chemical composition and quality of meat of wild and farm bred pheasants (Petkov 1984, Richter et al. 1992, Tucak et al. 2008, Hofbauer et al. 2010).

However, there are no studies on the quality of meat of pheasants, which orally ingest probiotics with feed and drinking water.

That is why our aim, in this study is to determine the impact some probiotics have on the quality of meat of pheasants.

MATERIALS AND METHODS

The experiment included 90 newly hatched pheasants (*Phasianus colchicus colchicus*), divided into 3 groups of 30 birds. They were raised under controlled microclimatic conditions, extended light period (24 h / day) and free access to food and water for 90 days. The pheasants received identical in composition and nutritional value standard compound feed for pheasants, balanced in protein, energy, amino acids, etc. as required by the National Research Council (NRC) (1994). Composition and nutritional value of the feed mixture are presented in Table 1.

Table 1.Ingredients and analyzed composition of feed mixtures

Ingredients, %	Starter (0-28 day)	Grower (29-90day)
Wheat+enzyme (10.5% crude protein)	49,45	61,16
Soybean meal (46% crude protein)	38	30
Fish meal (66% crude protein)	9	5
Sunflower oil	1,2	1
Synthetic L–lysine	--	0,1
Synthetic methionine	0,1	0,15
Synthetic treonine	--	0,06
Salt	0,1	0,18
Limestone	1,2	1,1
Dicalcium phosphate	0,4	0,8
Sodium bicarbonate	0,2	0,1
Aviax*	0,05	0,05
Micotox	0,1	0,1
Rovimix 11–C RonoP starter	0,2	0,2
Nutritive value	Starter (0-28 day)	Grower (29-90day)
Moisture,%	11,1	11,8
ME, (Kcal/kg)	2872	2912
ME (MJ/kg)	12	12,2
Crude Protein,%	28	24,1
Crude Fats,%	3,6	3,3
Linoleic acid,%	1,6	1,4
Crude Fiber,%	3,8	3,6
Crude ash,%	5,8	5,5
Ca,%	1,07	0,98
Available phosphorus,%	0,54	0,51
Phosphorus,%	0,84	0,8
Sodium,%	0,21	0,18
Chlorine,%	0,21	0,22
Chlorides,%	0,3	0,33
Lysine,%	1,7	1,41
Methionine,%	0,54	0,5
Methionine + Cysteine,%	1	0,93
Treonine,%	1,05	0,92
Tryptophane,%	0,35	0,3
Arginine,%	1,85	---

*to the combined forages of the control group is added Aviax 500g.kg-1 - which contains semduramicin sodium.

The experiment was conducted according to the following scheme (Table 2):

Group "A" (positive control) with antibiotic growth promoter Enrofloxacin and Colistin as a commercial product QUINOCOL (CEVA SANTE ANIMALE, France) in water (1 ml / 2 l of water); Second experimental group "B" - with the addition of probiotic CloSTAT® (Kemin, Inc., USA) in the feed (0,5 g / kg feed); third test group "C" - with the addition of probiotic Laktina® (Lactina, Bulgaria) in water (0,5 g / l of water).

Table 2. Scheme of experiment

Indexes	Control group (A)	Experimental group (B)	Experimental group (C)
Starter (0-28 day)	Combined forages for pheasants + prevention	Combined forages for pheasants + probiotic CloSTAT® in dose 0.5 g/kg*	Combined forages for pheasants + probiotic Laktina® – 0.5 g /l drinking water **
Grower (29-90day)	Combined forages for pheasants + prevention	Combined forages for pheasants + probiotic CloSTAT® in dose 0.5 g/kg*	Combined forages for pheasants + probiotic Laktina® – 0.5 g /l drinking water **

*-dosage of probiotic **CloSTAT®**- 0,5 g/kg (0,5 kg/t) is recommended by producer Kemin Industries, U.S.A.
- dosage of probiotic **Laktina® - 0,5 g/l drinking water) is recommended by producer Lactina, Bulgaria

Prevention:

• Antibiotic QUINOCOL® in drinking water at a dose of approximately 1 ml/2l of water per day (on the recommendation of the manufacturer) -from 1st to 5th day

• Vaccination against Newcastle vaccine CEVAC BI L® by instillation into the eye on the instructions for use of the vaccine, the 7th, 28th, 64th and 80th day

• Vaccination against Gumboro vaccine CEVAC GUMBO L® by drinking water on the instructions for use of the vaccine, the 14th and the 22nd day

• Vaccination against Avian Pox vaccine CEVAC FP L® by applying in the wing fold on the instructions for use of the vaccine-on the 56th day

Description of probiotics:

• Probiotic CloSTAT® (Kemin, Inc.) contains: spores *Bacillus subtilis* $2x10^7$ cfu/g, Maltodextrine, Calcium Carbonate

• Probiotic Laktina® (Lactina) contains: *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus casei*, *Bifidobacterium longum*, *Lactobacillus acidofilus* tbc in 1g not less than 1 billion.

Slaughtering technology

The pheasants had been slaughtered under the provisions of Council Directive 93/119/EC (1993). The birds were stunned by a blow on the occipital region of the head and killed by subsequent bleeding.

Physical and chemical tests

Laboratory analysis to establish the quality of the meat of pheasants were performed separately for breast and leg muscles in all three experimental groups.

Samples were taken from the pectoral muscles (breast) and femoral muscles (leg).

The muscle was separated from the bones and the skin and subcutaneous fat were also removed.

For characterizing the meat quality the following physical and chemical parameters were examined: pH value, colour, water holding capacity (WHC), water content, myoglobin, total protein and ash content.

Measurement of the active acidity (pH) was performed 24 h post mortem, according to ISO 2917:1999. pH meter HANNA instrument HI 8314 equipped with a thermometer and electrodes was used.

The indicator colour (R/525nm) was determined using spectrophotometer Specol 11 (equipped with a plug-in colour). Water holding capacity (WHC) was determined by the method of Grau and Hamm (1953) and expressed as content of free water in percentages.

The moisture content was determined according to BDS 5712: 1974.

Myoglobin content (mg/kg) by Hornsey (1956).

The protein content was determined by BDS 9374: 1982, and ash content - BDS 9373: 1980. The data was processed statistically by the Program StatMost 3.6, Dataxiom Software, 2003. The results were calculated using five replicates (n = 5) and presented as the average (mean) with a standard deviation (SD) (the SD is calculated for the group of 5 replicates).

The significance was defined as low (p≤0,05); average (p≤0,01) and high (p≤0,001) degree.

RESULTS AND DISCUSSIONS

The results of the studied physical and chemical parameters of breast and leg muscles of pheasants are presented in Table 3.

Average mean pH values in meat from the breasts vary between 5.55 and 5.74, the differences between groups are significant.

Franco et al. (2013) obtained identical average pH(5, 69) in breast muscle of pheasant grown extensively and Kokoszyn'ski et al. (2012)

Table 3
Physical and chemical characteristics of breast and leg meat of pheasants

Physical and chemical characteristics of breast and leg meat of pheasants				
Parameter (n=5) mean±SD	**Type of meat**	**Control group A**	**Experimental group B**	**Experimental group C**
pH	breast	5,553^{b}** ±0,1008	5,607^{a}* ±0,1151	5,744^{a}* ^{b}** ±0,0812
	leg	6,511 ±0,0970	6,464 ±0,1539	6,513 ±0,2097
Color, 525nm/R	breast	38,7043 ±0,8529	38,810 ±0,9938	39,490 ±0,9329
	leg	37,044^{ab**} ±0,3393	38,471^{a}** ±1,1087	38,606^{b}** ±1,0910
WHC, % free water	breast	29,524 ±1,9099	27,556 ±4,4274	27,441 ±2,0141
	leg	19,620 ±2,6846	20,571 ±2,1765	20,413 ±1,9630
Moisture, %	breast	72,243 ±0,6480	72,313** ±0,2843	71,904** ±0,1474
	leg	74,764* ±0,2017	75,057* ±0,2133	74,893 ±0,1475
Myoglogin, mg/кg	breast	0,971 ±0,1469	1,013* ±0,1525	0,856* ±0,0489
	leg	2,577* ±0,2597	2,380 ±0,3878	2,234* ±0,2342
Protein, % of total mass	breast	25,614 ±0,6989	25,533** ±0,3630	25,979** ±0,1671
	leg	22,680 ±0,5879	22,213** ±0,0881	22,624** ±0,3349
Ash, % of total mass	breast	1,244 ±0,0355	1,247 ±0,0269	1,217 ±0,0461
	leg	1,147^{a**} ±0,0248	1,156^{b}** ±0,0270	1,200^{ab}** ±0,0224

*- p≤0,05; **- p≤0,01; ***- p≤0,001

slightly higher pH (5.80) in the breasts of Mongolian x Versicolor cross-bred.

In the breast muscle of the groups receiving probiotics (B and C) higher pH values were obtained in comparison to the control group (A) whose feed contained antibiotic QUINOCOL.

Fatma (2010) received similar results, measured the pH of breast muscle of broilers 24 hours after slaughter, and demonstrated a statistically significant difference between pH of the meat samples from chickens which had received probiotics in the food and chickens which had not.

Aksu et al. (2005) in a similar experiment with the addition of probiotics (*S. cerevisiae*) in the broiler feed found that the use of probiotic leads to an increase of pH in the meat from the breasts and leg.

Different results were obtained by Ivanović et al. (2012) about pH in the leg and breast of broilers, measured 5 hours after slaughter. According to the authors the probiotic was added to the diet of a test group which led to lower pH, in contrast to probiotics added to the food of the other test group which showed increased pH values compared with the control group, with statistically significant difference.

In our results , as opposed to the breast muscle, in the leg muscle significant difference between groups was not observed, the pH was in the range 6.46 to 6.51.

In all groups of experiments, the level of pH at the leg muscle on average is 0.86 units higher than that measured in the breast muscles.

These results are in accordance with studies of Richter et al. 1992, Kuzniacka et al. 2007, Paulsen et al. 2008, Hofbauer et al. 2010.

This fact can be explained by the different type of myofibres in the studied muscles. Breasts muscles of pheasants are composed mostly (> 70%) of fast-twich, glycolytic fibres (IIB type), while the muscles of the legs are with a higher percentage of anoother glycolyticoxidative (IIA) or oxidative fibre types(Kiessling 1977). This difference is common to Gallinaceous birds and affects the ability to scramble at the expense of short-haul flight (Pyörnilä et al. 1998).

Stress from manipulations before slaughter may also have an impact, especially in the breast muscles which contain much more IIB fibers (Lawrie and Ledward 2006).

The absence of differences in the pH of the leg muscle and the higher values of pH, in comparison to the breast muscle may be due to smaller glycogen reserves in the leg muscles after the slaughter of pheasants (Franco et al., 2013), which in our opinion is a result of earlier puberty, altered behavioural responses and expressed aggressiveness.

No statistically significant differences in WHC between the experimental and control groups were observed.

No significant differences in WHC and cooking loss (CL) are the results of Pelicano et al. (2003) who tested three different types of probiotics in broilers chicken.

Kim et al. (2010) found a positive influence of probiotics used in fattening pigs on WHC of meat and improving its technological and cooking qualities (tenderness, flavour characteristics, etc.).

The higher percentage of free water in the breast muscles for all groups of our experiment compared to the leg muscles can be explained by the low pH of this type of muscle.

Loss of water is higher in muscles with a low pH as shared by other authors (Hofmann, 2004).

The tested probiotics have influenced the colour of breast meat, with higher values for the test group that received probiotic Laktina® in water) 39.49 at $\lambda = 525$ nm.

However, differences between groups are not proved, unlike the meat colour in the leg, where we have significant difference ($p \leq 0{,}01$) between the experimental and control group.

The observed differences in colour correspond with the content of myoglobin in red meat - significantly at the lowest in the test group (2,234 mg / kg) where the meat colour is lighter.

The result was the same, in the breast muscles, where the lightest coloured birds stand out from the test group (0,856 mg/kg) ,although not significantly.

While testing the three different types of probiotics in broiler chickens Pelicano et al. (2003) found that the concomitant use of probiotics in feed and drinking water significantly reduces the values of lightness in the colour of breast muscles after slaughter, leading to "a little pale" meat and values of the red component where redness were higher in the treated with probiotics groups than in the control group., Meng et al. (2010) also obtained the same results for colour and

higher values of the red component (redness) in pigs fed with probiotics compared with pigs fed without probiotics.
The values obtained for water content in the breast muscle in all groups were lower than the values in the leg muscle.
This corresponds with the results of other authors (Severin et al. 2007, Hofbauer et al. 2010, Franco et al., 2013), who received approximately 71.8 to 73.09% of moisture for breast muscle, and from 74.2 to 75, 2% moisture for the leg muscle of pheasants.
There were significant differences in water content between treatment groups B and C (which received various probiotics) in samples of breast meat.
There was significant difference ($p \leq 0,05$) for the samples of leg meat between the experimental and the control group (C) that received CloSTAT® in feed.
There were no significant differences in water content in group (C) that received probiotics in the drinking water. Protein content of the meat from the breasts of all groups of pheasants was higher than the content in the leg muscle.
Same results were obtained by Hofbauer et al. (2010) and Franco et al. (2013), where the protein concentrations in breast muscles were significantly higher than those in the muscles of the legs.
Same are the conclusions of Tucak et al. (2008), although Severin et al. (2007) found no difference between the protein content of breast and leg muscle of pheasants.
A high protein content is associated, by most authors, with a low content of moisture and fat in this type of muscle.
It is also important to note that the protein content that we found for the breast meat of pheasants (25.5 to 25.9%) is higher than that found in broiler chickens (Ding et al., 1999; Qiao et al ., 2002), which varies between 22.6 and 24.7%.
The protein content in the leg is higher 22.2 -22.6%) compared to the findings of Ivanović et al. (2012) in broilers - 19.83%.
Both probiotics affected the protein content in meat from the legs and breasts differently.
This confirms the assumption that probiotics affect the protein metabolism.
The results show no significance compared to the control group, a slightly higher amount of protein in the breast muscle of test group (C) and slightly lower - in the leg muscle of test group (B).
The opinions of other scientists on this issue are different.
Higher protein synthesis is established by Ignatova (2004), in experiments with broiler chickens.
Ivanović et al. (2012) and Hossain et al. (2012) found higher levels of protein in breasts and less in the leg, using probiotics also in broilers. Furthermore, according to Hossain et al. (2012) the addition of probiotic increases the absolute and relative weight of the breasts.
Again after using probiotics for broilers, Sazedul et al. (2010) found higher protein content in the meat from the leg (respectively 23.89 and 21.94%).
In terms of mineral composition, no difference in samples of breast meat was observed.
Values in all groups are close to those established by Petkov (1999) 1,08 - 1,23%, Franco et al. (2013) 1,26%, Hofbauer et al. (2010) from 1.30 to 1.39% of mineral substances in breast muscle of pheasants.
In the leg meat there were significant differences ($p \leq 0,01$) in the mineral composition of group (C) compared to the control and also between the two experimental groups (B and C) to which a probiotic was given.
The data correlated with the measured values by Petkov (1999) from 1.02 to 1.18%, and Tucak et al. (2008) from 1.06 to 1.15% for mineral substances in the leg muscles of pheasants.

CONCLUSIONS

The results obtained in these studies on antibiotic growth promoters and their ecological alternatives - probiotics, give us reason to form the following conclusions:

• The use of probiotics (CloSTAT® and Laktina®) orally in pheasants results in an increase of pH in the breast muscle, lighter meat color and lower amount of myoglobin in the leg and breast muscles.

• The use of the probiotic CloSTAT® in the feed resulted in an increase of water content in the leg muscle of pheasants.

• The use of the probiotic Laktina® in drinking water results in an increase of the mineral substances in the leg muscle of pheasants.

• Probiotics (CloSTAT® and Laktina®) impact differently on protein metabolism in breast and leg muscles of pheasants. The use of Laktina® leads to the accumulation of large amount of proteins in the breast muscle, and the use of CloSTAT® - reduces their amount in the leg muscle.

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