Effect of different probiotics on breast quality characteristics of broilers under Salmonella challenge

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Abstract

The current study was performed to investigate the influence of probiotics or antibiotic on breast quality characteristics of broiler chickens that were subjected to Salmonella challenge. Two hundred, one-day-old Cobb 500 chicks were allocated in five experimental treatments for 42 d. Ten cages of birds received control (+CONT), unsupplemented, unchallenged; T1=positive antibiotic neoxyval (NEOX), challenged; T2=negative control (-CONT), unsupplemented, challenged; T3=supplemented with probiotic Toyocerin (TOYO), challenged; and T5=supplemented with probiotic CloSTAT™ (CLOS), challenged. Birds in treatments T2 to T5 were challenged with 3x10^9 CFU/mL of Salmonella enterica subsp. typhimurium on day 16. Nine birds per treatment were sampled at the end of the trial for breast characteristics. Overall, pH and temperature values of the breast muscle were similar among all groups tested. Cooking loss results indicated that breasts from T3 birds had the highest degree of shrinkage upon cooking while those of the probiotic group had similar control values (P<0.0001). Probiotic supplementation reduced the extent of destruction of myofibrils caused by homogenisation (P<0.0001). Warner-Bratzler shear test and texture profile analysis showed that neither treatments nor Salmonella challenge had any negative impact on texture or sensory attributes of chicken breast. In conclusion, results show that breast characteristics were better when probiotics were supplemented in the diets.

Introduction

Recently there are increasing concerns about the risk of developing cross-resistance and multiple antibiotic resistances to pathogenic bacteria, which could result in proliferation of antibiotics-insensitive bacteria; this could lead to a decrease in the therapeutic effectiveness of antibiotics used by humans (Castanon, 2007; Maiorano et al., 2012). Probiotics, prebiotics and synbiotics are among the alternative growth promoters that are already used in practice (Teo and Tan, 2006; Maiorano et al., 2012). Fuller (1989) defined probiotics as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Probiotics are reported to prevent the colonisation of the gut by pathogenic bacteria like Clostridium perfringens and Salmonella through competitive exclusion (Teo and Tan, 2005, 2006; Abudabos et al., 2013). Other reports showed that probiotic improves the performance of broiler chickens by promoting higher growth rate and feed conversion efficiency through different mechanisms (Rajput and Li, 2012). The results on the effect of probiotics on carcass quality were controversial and many unclear results have been presented. In addition, most of the reviewed researches focused on the role of probiotics in animal performance and immune response, rather than the quality of the product under challenged situation. Some authors reported advantages of probiotic administration on meat quality in relation to colour, pH and tenderness (Pelicano et al., 2003; Kabir, 2009), while others did not observe improvements when probiotics were used (Owings et al., 1990). On the other hand, scanty data are available on the effect of probiotic on meat quality when the birds are subjected to bacterial challenge. Breast muscle provides the greatest portion of edible meat in broilers and the contribution of breast muscle to total carcass meat is extensive. Any improvements in the characteristics of the breast can be considered meaningful in terms of carcass quality and consumer preferences. For this reason, it is always necessary to perform evaluation as regards to yield, chemical composition and properties of the breast muscle (Abeni and Bergoglio, 2001).

Accordingly, the objective of the present study was to examine the effect of probiotic or antibiotic supplementation on breast quality of broiler chickens that were subjected to Salmonella enterica subsp. typhimurium challenge.
Diets and treatments

Typical isocaloric and isonitrogenous starter (0 to 14 d) and finishing (15 to 42 d) diets based on corn soybean meals were formulated in the mash form which met or exceeded the recommendations in commercial practice in Saudi Arabia; the diets were mixed according to Table 1. The chicks received one of 5 treatments randomly as follows: T1=positive control (+CONT), unsupplemented, unchallenged; T2=negative control (-CONT), unsupplemented, challenged; T3=supplemented with antibiotic neoxoval (NEOX), challenged; T4=supplemented with probiotic Toyocerin (TOYO), challenged; and T5=supplemented with probiotic CloSTAT™ (CLOS), challenged. Toyocerin® (Toyocerin, Barcelona, Spain) was used as the reference antibiotic; 1 g of it contains 200 mg oxytetracycline and 200 mg neomycin. Toyocerin® is a product with an active ingredient consisting of viable spores of Bacillus cereus var. toyoi (Rubinum, Barcelona, Spain). CloStat® is a probiotic supplied by Kemin Industries Inc. (Des Moines, IA, USA); it contains a unique strain of Bacillus subtilis PB6 that inhibits the proliferation of Clostridium.

Challenge inocula

On day 16, the birds in treatments 2 to 5 were challenged with Salmonella enterica subsp. typhimurium (3×10^9 CFU/mL). Chicks were gavaged with 1 mL of cocktail containing 3×10^9 CFU/mL Salmonella enterica which was obtained commercially (MicroBiologics, St. Cloud, MN, USA).

The strain chose for this experiment was Salmonella typhimurium ATCC13311, which was used following the procedure described by Marq et al. (2011). According to Chalghoumi et al. (2009) this strain is known to colonise broilers efficiently. Organisms were retrieved from suspensions stored at -80°C and plated two times successively on tryptone soy agar (CM 129; Oxoid Ltd., Basingstoke, UK) for 24 h at 37°C. Then, 10 mL of preculture was prepared by picking a single colony into a sterile preewarmed tryptone soy broth and incubated at 37°C for 24 h. Subsequently, 5 mL of Salmonella typhimurium preculture was transferred into 150 mL of TSB and incubated with orbital shaking at 37°C for 18 h. The challenge inocula were prepared by diluting the Salmonella typhimurium suspension appropriately in sterile TSB to give final viable cell concentrations of 3×10^9 CFU/mL; the inocula were then aliquoted, stored at 4°C, and rapidly used for the oral infection. Counts of viable cells were determined both before and after the inoculation of chicks.

**Measurements**

At days 40, 41 and 42, three birds were selected per treatment/day, for a total of 9 birds per treatment selected for measuring meat characteristics. After euthanasia, the jugular vein was cut, feather, heads and shanks were removed and the remaining carcasses were dissected. The left and right breasts from each bird were used for the quality measurements. The pH of the breast muscle was measured at 15 min post-mortem using a microprocessor pH-meter (Model pH 211; Hanna Instruments, Cloud, MN, USA). The pH of the breast muscle was measured at 15 min post-mortem using a microprocessor pH-meter (Model pH 211; Hanna Instruments, Cloud, MN, USA).

**Table 1. Dietary ingredients and chemical composition of starter and finishing diets.**

| Ingredients, % | Corn | Soybean meal | Palm oil | Dicalcium phosphate | Limestone | Salt | Vitamin-mineral premix | DL-Methionine | Lysine-HCL | Threonine | Sodium bicarbonate | Choline chloride | Neoxyl | Toyocerin | Clostat | Total | Chemical composition |
|----------------|------|--------------|---------|---------------------|-----------|------|-----------------------|--------------|------------|----------|---------------------|--------------|--------|-----------|--------|-------|---------------------|
| T1, T2         | 63.01 | 31.15        | 1.72    | 1.96                | 0.73      | 0.25 | 0.50                  | 0.18         | 0.18       | 0.07     | 0.12                | 0.05         | 0.00   | 0         | 0      | 100   | 3000                |
| T3             | 63.01 | 31.15        | 1.72    | 1.96                | 0.73      | 0.25 | 0.50                  | 0.18         | 0.18       | 0.07     | 0.12                | 0.05         | 0.00   | 0         | 0      | 100   | -                   |
| T4             | 62.91 | 31.15        | 1.72    | 1.96                | 0.73      | 0.25 | 0.50                  | 0.25         | 0.25       | 0.07     | 0.12                | 0.05         | 0.00   | 0         | 0      | 100   | -                   |
| T5             | 62.91 | 31.15        | 1.72    | 1.96                | 0.73      | 0.25 | 0.50                  | 0.25         | 0.25       | 0.07     | 0.12                | 0.05         | 0.00   | 0         | 0      | 100   | 3150                |
| T1, T2         |       |              |         |                     |           |      |                       |              |            |          |                     |              |        |           |        |       | 17.3                |
| T3             |       |              |         |                     |           |      |                       |              |            |          |                     |              |        |           |        |       | 4.97                |
| T4             |       |              |         |                     |           |      |                       |              |            |          |                     |              |        |           |        |       | 0.51                |
| T5             |       |              |         |                     |           |      |                       |              |            |          |                     |              |        |           |        |       | 0.98                |
| T1, T2         |       |              |         |                     |           |      |                       |              |            |          |                     |              |        |           |        |       | 0.80                |
| T3             |       |              |         |                     |           |      |                       |              |            |          |                     |              |        |           |        |       | 0.73                |
| T4             |       |              |         |                     |           |      |                       |              |            |          |                     |              |        |           |        |       | 0.85                |
| T5             |       |              |         |                     |           |      |                       |              |            |          |                     |              |        |           |        |       | 0.85                |
| T1, T2         |       |              |         |                     |           |      |                       |              |            |          |                     |              |        |           |        |       | 0.41                |

*(T1, treatment 1 (positive control, unsupplemented, unchallenged); T2, treatment 2 (negative control, unsupplemented, challenged); T3, treatment 3 (supplemented with antibiotic neoxoval, challenged); T4, treatment 4 (supplemented with probiotic Toyocerin, challenged); T5, treatment 5 (supplemented with probiotic). °Vitamin-mineral premix contains the following per kg: vitamin A, 2,000,000 U; vitamin D, 1,000,000 U; vitamin E, 16,000 U; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B2, 1600 mg; vitamin B3, 1000 mg; vitamin B6, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin, 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400 mg; iron, 1200 mg; manganese, 18,000 mg; selenium, 60 mg; zinc, 14,000 mg.)*

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Woonsocket, RI, USA). Two readings were taken and the mean value was calculated. The core temperature in breast muscle was measured at 15 min post-mortem with a portable digital thermocouple (EcoScan Temp JKT; Thermo Scientific, Waltham, MA, USA). The mean of two readings was calculated to determine final temperature. The colour values of CIELAB Color System – L*(lightness) a* (redness) and b* (yellowness) – were determined on the breast muscles 15 min and 5 h after slaughtering according to Commission International de l'Eclairage (1976) using a Chroma meter (CR-400; Konica Minolta, Tokyo, Japan). Then, breasts were stored at -80°C until the time of further analysis for other meat quality measurements. At the time of the analysis, the frozen muscles were thawed overnight in chiller at 4°C. The myofibril fragmentation index (MFI) of the breast muscle was determined as described by Culler et al. (1978). A 4-g muscle sample was scissored, minced, and then it was homogenised (Ultra Turrax) with 40 mL of a cold isolating MFI buffer. The absorbance of 0.5 mg/mL solution was determined at 540 nm, then MFI was determined by multiplying the absorbance value by the dilution factor.

The water-holding capacity (WHC) was determined based on the technique described by Haman (1960) and following the modification performed by Wilhelm et al. (2010). Two replicates of around 2 g were collected from the cranial side of the breast fillets of each sample and cut into cubes. Then, the sample was placed between two filter papers and two Plexiglas and left under a 10-kg weight for 5 min. Afterwards, the sample was weighed and WHC was determined as the difference between the initial and final weights using the following formula: \[100 - \left(\frac{W_i - W_f}{W_i}\right) \times 100\], where \(W_i\) and \(W_f\) are the initial and final sample weights, respectively.

The drip loss (DL) was determined in the broiler breasts by weighing the samples 20 min after slaughter, then kept in plastic bags and left in a cooler (4°C) for 24 h. The fillets were re-weighed after that to determine the DL values as a percentage based on the initial sample weight.

For measurements of cooking loss (CL), *P. major* muscle samples were placed in a commercial indoor countertop grill and cooked at an internal temperature of 70°C. The temperature was monitored by inserting a thermocouple thermometer probe (Ecoscan Temp JKT; Thermo Scientific) into the geometric centre of the muscle. Cooking loss percentage was determined as the difference between the initial and final weights and expressed as percentage of the initial weight. Cooked samples were then used to evaluate shear force (SF) according to the procedure described by Wheeler et al. (2005). After samples were cooled to room temperature (21°C), three round cores (1.27 cm in diameter) were removed from each muscle sample parallel to the longitudinal orientation of the muscle fibres. Cores were obtained using a handheld coring device. Shear force was determined as the maximum force (kg) perpendicular to the fibres using Texture Analyzer (TA-HD; Stable Micro Systems, Godalming, UK) equipped with a Warner-Bratzler attachment. The crosshead speed was set at 200 mm/min.

To determine the texture profile analysis (TPA), the samples of breast muscles were cooked as described previously, and then samples were obtained by scoring the muscles parallel to the longitudinal direction of the muscle fibre with the aid of a handheld corer. The TPA was done using Texture Analyzer (TA-HD; Stable Micro Systems) equipped with a compression platen attachment. Each sample underwent 2 cycles of 80% compression. The variables determined were: hardness (the maximum force needed to compress the sample), cohesiveness (a ratio between the total energy required for the first and second compression), springiness (the ability of a sample to recover to its original form after removing of the compressing force), and chewiness (a result of springiness×hardness×cohesiveness).

### Statistical analysis

All statistical analysis was performed using the Statistical Analysis System (SAS, 2003). Five treatments were arranged in 10 replications in a randomised complete block design; when applicable repeated measures were used to investigate the time effect. Means for measurements showing significant differences in the analysis of variance were tested using the PDIFF option. The overall level for statistical significance was set at P<0.05. All values were expressed as statistical means±standard error of mean.

### Results and discussion

The treatment had no effect on pH value when measured 15 min post-mortem (Table 2). It was reported that pH value varied considerably within post-mortem time period ranging from 5.78 to 6.59 at 15 min (Dodge and Peters, 1960). The change in pH is one of the most significant changes that occur during rigor mortis and can affect meat quality characteristics such as texture, colour and water holding capacity (Mehaffey et al., 2006). Fletcher et al.

### Table 2. pH, temperature and colour parameters of breast muscles measured at different times.

|        | pH     | Temperature, °C | L*     | a*       | b*       |
|--------|--------|-----------------|--------|----------|----------|
|        | 15 min | 15 min          | 15 min | 5 h      | 15 min   | 5 h      | 15 min   | 5 h      |
| Group  |        |                 |        |          |          |          |          |
| T1     | 6.72   | 27.3            | 44.2   | 49.4     | 3.4ab    | 3.1      | 5.9      | 6.8      |
| T2     | 6.78   | 28.1            | 45.5   | 50.1     | 2.9ab    | 2.9      | 3.2      | 8.2      |
| T3     | 6.87   | 28.1            | 48.5   | 50.8     | 2.2ab    | 2.2      | 3.4      | 6.5      | 7.5      |
| T4     | 6.96   | 27.7            | 45.0   | 51.6     | 3.6ab    | 3.6      | 3.5      | 7.1      | 7.8      |
| T5     | 6.86   | 28.2            | 45.8   | 49.7     | 2.7ab    | 2.7      | 4.2      | 6.9      | 9.6      |
| Probability | 0.07 | 0.34 | 1.03 | 0.18 | 0.04 | 0.45 | 0.51 | 0.71 |

L*, lightness; a*, redness; b*, yellowness; T1, treatment 1 (positive control, un-supplemented, un-challenged); T2, treatment 2 (negative control, un-supplemented, challenged); T3, treatment 3 (supplemented with antibiotic neoxyval, challenged); T4, treatment 4 (supplemented with probiotic Toyocerin, challenged); T5, treatment 5 (supplemented with probiotic). Each mean is based on measurements from 9 birds per treatment at the end of the trial. *Means in the same column with different superscripts differ significantly. *P<0.05; ns, not significant.
(2000) reported that pH measurements after 15 min post-mortem were a good indicator of meat characteristics. Generally, a rapid post-mortem pH decline in breast meat can lead to protein denaturation that may consequence in pale colour and low WHC. The data presented in this trial are higher than those of Battula et al. (2008) and Corzo et al. (2009) who reported that, on average, pH after 15 min was between 6.3 and 6.6 for broiler breast meat. In this trial the lowest pH value obtained after 15 min was 6.72, which suggested that neither probiotics nor bacterial challenge had any negative impact on the pH of the breast muscle. The treatment had no effect on breast temperature value when measured 15 min post-mortem (P>0.05).

The data related to breast colour after 15 min and 5 h post-mortem are presented in Table 2. Lightness (L*) and yellowness (b*) values of the breast were not affected by treatment 15 min post-mortem. On the other hand, values of a* (redness) were lower (P<0.05) in NEOX group (2.2) than in CONT group (3.4) or TOYO group (3.6) 15 min after slaughter. Lightness, redness and yellowness values of the breast were not affected by treatment after 5 h (P>0.05).

Cooking loss is a measure of water percentage lost upon cooking as a result of shrinkage. The degree of shrinkage upon cooking is directly correlated with loss of juiciness to the palate. In this trial, the CL of breast muscles was influenced by treatment (P<0.0001), as shown in Table 3. The CL percent was the highest from breast obtained from NEOX group (33.8%) followed by +CONT (23.3%), while it was the lowest for CLOS and -CONT (18.2 and 18.7%, respectively), and intermediate for the TOYO group (21.4%). Water loss generally is associated with reduction in the nutritional value of the meat since some nutrients are lost; as a result meat becomes less tender. Both DL and WHC were not influenced by the treatment (P>0.05). Water holding capacity is a term used to describe the ability of muscles to bind water under a specific set of conditions. Generally, it increases with age of the birds, since the increase of the muscle fat content leads to an increase in WHC and a decrease in CL percentage (Corzo et al., 2009).

The MFI of the breast muscle was influenced by treatment (P<0.0001) as shown in Table 3. The MFI increased in -CONT, NEOX and +CONT groups as compared to the groups which were supplemented with probiotics (CLOS and TOYO). Myofibrillar fragmentation is the extent of destruction of myofibrils caused by homogenisation. Olson et al. (1976) reported that MFI values are correlated with other indices in the muscle such as SF and tenderness. It can be concluded that probiotic supplementation caused less damage to the myofibrils.

The SF value was not influenced by treatment (Table 3). Pelicano et al. (2003) estimated the shearing force values in breast muscle between 5.5 to 5.8 kg/f/g. Therefore, the treatments in this trial did not affect meat tenderness since SF values were between 2.71 to 3.31 kg and almost 50% less than the values reported by Pelicano et al. (2003). In a subsequent study, Pelicano et al. (2005) examined the effects of Lactobacillus and Bacillus subtilis-based probiotics on Warner Bratzler shearforce and reported a value of 3.88 and 4.08 kgf which was similar to the control.

Table 3. Physical properties of breast muscle meat of broilers fed experimental diets.

| Group      | CL, % | DL, % | WHC, % | MFI | SF, kgf |
|------------|-------|-------|--------|-----|---------|
| T1         | 18.7b | 1.78  | 36.5   | 112.2bc | 2.71   |
| T2         | 23.3b | 1.86  | 33.5   | 101.1bc | 2.81   |
| T3         | 33.8b | 2.25  | 37.4   | 101.2bc | 3.31   |
| T4         | 21.4b | 1.75  | 39.5   | 73.4bc  | 3.18   |
| T5         | 18.2b | 2.10  | 38.1   | 51.1bc  | 3.20   |

The CL, cooking loss; DL, dripping loss; WHC, water-holding capacity; MFI, myofibril fragmentation index; SF, shearing force; T1, treatment 1 (positive control, unsupplemented, unchallenged); T2, treatment 2 (negative control, unsupplemented, challenged); T3, treatment 3 (supplemented with antibiotic neosyn, challenged); T4, treatment 4 (supplemented with probiotic Toyokem, challenged); T5, treatment 5 (supplemented with probiotic). Each mean is based on measurements from 10 breasts per treatment. ***Means in the column with different superscripts differ significantly. ***P<0.0001; ns, not significant.

Table 4. Texture profile analysis in breast meat of broilers.

| Group | Hardness, kg | Springiness | Cohesiveness | Chewingness |
|-------|--------------|-------------|--------------|-------------|
| T1    | 3.7          | 0.61        | 0.45         | 0.10        |
| T2    | 3.7          | 0.58        | 0.42         | 0.14        |
| T3    | 4.3          | 0.61        | 0.44         | 0.12        |
| T4    | 5.3          | 0.63        | 0.47         | 0.15        |
| T5    | 3.8          | 0.64        | 0.44         | 0.13        |

TPA, texture profile analysis. Each mean is based on measurements from 18 breasts per treatment. ns, not significant.

Conclusions

In this trial pH and temperature values of the breast muscle were similar among all groups tested and neither probiotics nor bacterial challenge had any negative impact on the pH or temperature. The CL results indicated that breasts from broilers received the antibiotic (NEOX) had the highest degree of shrink-
age upon cooking as compared to other treatments, while those received the probiotic had similar values to the control. Probiotic supplementation reduced the extent of destruction of MFI caused by homogenisation. Warner Braitzer shear test and TPA showed that neither the treatments nor the challenge had any negative impact on texture or sensory attributes of chicken breast. The redness (a*) of the breast muscle was lower in NEOX group as compared to the CONT or TOYO groups at 15 min post-mortem. According to the results of this trial, it can be concluded that probiotic supplementation moderately influenced the characteristics of the broiler breast muscle.

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