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"Probiotics Feed Supplement" to Improve Quality of Broiler Chicken Carcasses

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Abstract: This work was carried out to evaluate the carcass keeping quality and microbial content of broiler chicken following the use of different probiotics (*Bacillus subtilis* and zinc bacitracin) as feed supplement. A total number of 270 one day old chicks reared under the same conditions of poultry housing, they divided into three groups. The first group was used as a control group. The second group was fed on diet mixed with *Bacillus subtilis* spores The third group fed on diet mixed with zinc bacitracin). All chickens (3 groups) were separately sacrificed at slaughter plant on age of 45 days. Broiler carcasses were kept in refrigerator and examined for keeping quality parameters (pH, water holding capacity, TBA, cooking loss, shear force and instrumental color); followed by microbiological examination for Aerobic plate count at 35°C, Coliforms (MPN), *Staphylococcus aureus* count and isolation of each of Salmonellae, *Campylobacter jejuni*, *Yersinia enterocolitica* and *E. coli*. Results showed that, the pH, TBA and cooking loss of the treated samples were significantly lower than the control. Water holding capacity and shear force varied significantly ($P<0.01$) as compared with the control group. The color of the breast muscle was lighter in the treated groups and was darker in the control. Aerobic plate count at 35°C, Coliforms (MPN) and *Staphylococcus aureus* count were significantly lower ($P < 0.05$) in probiotic treated chicken as compared with the control. The incidence of Salmonellae, *C. jejuni* and *Yersinia enterocoilitica* were significantly lower ($P < 0.05$) in chicken carcasses fed on diet mixed with probiotics, but *E. coli* was not significantly differ than control. In conclusion,addition of probiotics as feed supplement were recommended to improve the keeping quality parameters and the microbial status of carcass in broiler chicken

key words: Probiotics · C. jejuni · Salmonellae · Yersinia enterocolitica · pH · TBA · Cooking loss · Shear force · Instrumental colour

INTRODUCTION

Broilers industry has been looking for using natural additives for the continuous improving of the final quality of products and consequently quality Indexes. The most important alterations directly related to chicken meat quality were pre and post slaughter practices, bird age, strain, sex, environment and nutrition and within the latter antibiotics have been particularly considered by international health [1]. A world trend to reduce the usage of antibiotics in animal feed due to residues problems in the final products was evident. Most of broilers industry practioners have been given a growth promoter as an additive in ration [2, 3]. Probiotics are alive microbial feed supplement which have beneficially affects on the host animal by improving its intestinal microbial balance. These probiotics organisms should be non pathogenic, non toxic and also resistant to low pH and bile salts to improve its chances of survival in the gastrointestinal tract [4, 5]. The probiotic strains used in poultry industry are belonging to aerobic spore forming bacteria, genus Bacillius. The performance of broiler supplemented with *Bacillius coagulans* as probiotic was early detected [6]. A recent method for preparation of probiotic for poultry industry was described by Beliavskaia *et al.* [7]. The spores from number of different *Bacillis species* obtained by heat and ethanol treatment of fecal material from organically reared broilers followed by aerobic plating are currently used as human and animal probiotic. The use of probiotic for carcass quality improvement has been questioned and many unclear results have been shown. Some authors reported advantages of probiotic administration [8-12]. Other studies didn't observe improvement when probiotics were used

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[13,14]. Therefore, the aim of this study was to evaluate the effect of *Bacillus subtilis* and zinc Bacitracin as probiotics feed supplement on physical and bacteriological quality of broiler carcasses.

MATERIALS AND METHODS

Chicks: A total number of 270 one day old, Cobb chicks was purchased from Poultry Company, at Beni-Suef, Egypt.

Used Probiotics

Bacillus Subtilis: *B. subtilis* were used as 4 x 10^5 CFU/ g product (Megalo, Amoun Man. Add El-obour city,Cairo, Egypt) and added to the diet following manufacturer's instructions.

Zinc Bacitracin: Zinc bacteracin was used as 500mg/g product (Baciferm, Mena Vet Saddat city Industerial Area, Egypt) and added to the diet following manufacturer's instructions.

Experimental Design: A total of 270 one day old Cobb chicks, was divided into two treated groups (plus the control group.

The experiment was triplicate with 30 birds in each trial. All groups were prophylactically vaccinated according to the local routine vaccination programe. Chicks were divided into 3 pens (2.75 x 1.4 m) in the experimental poultry house, faculty of veterinary medicine, Beni-Suef university. There were 30 birds /pen for a final density of 8 birds /m^2. Chicks were reared under good hygienic conditions (under day light and regular fan).The first group was provided with feed and water *ad-libitum* and kept as the control group. The second group was fed on the balanced diet supplemented with probiotic I (*Bacillius subtilies; 4 x10^5 colony forming units CFU/g product*), *Bacillius subtilis*-based probiotic was added to the diet in a proportion of 1.5 kg/ton diet allover the period of rearing (1-45 days of age). The third group was fed on the same diet supplemented with probiotic II (zinc Bacitracin 500 mg/g product). Zinc Bacitracin was added to the diet in a proportion of 1kg/ton diet allover the period of rearing (1-45 days of age). At the end of experiment, all groups were separately sacrificed in a poultry slaughter-plant. Carcasses were rapidly transferred in sterile ice-box to Food Hygiene Laboratory, Faculty of Veterinary Medicine, Beni-Suef University. Carcasses were chilled and examined every two days till signs of spoilage appeared for the following:

Phyiscal Examination

pH: pH was directly determined using digital pH meter in breast muscle. The measurements were done 24 hours after slaughtering(ultimate pH) and every two days during chilling till signs of spoilage appeared according to the technique described by Allen *et al.* [15].

Water Holding Capacity: The method described by Hamm [16] was used for evaluation of water holding capacity. Meat cube of 0.5g was placed between two filter papers covering two glass plates for 5 minutes and a 10 kg weight was placed on the top of glass plates for 5 minutes. The differences in breast muscle weights before and after the procedure represented the water loss. The results were expressed as percentages of fluid exudates in relation to the initial sample weight.

Thiobarbituric Acid Reactive Substances (TBA): TBA value was determined according to the method described by Vyncke [17].

Cooking Loss and Shear Force: Cooking loss was determined in an oven whereas samples were weighed and then cooked in oven at 180°C for 20 minutes with the internal temperature of breast muscle 75°C. Samples were cooled at room temperature, reweighed. Cooking loss was calculated as the differences between the initial and final samples weights. Samples were chilled at refrigerator temperature overnight and used for determination of tenderness. The shear force was measured with a blade 168mm wide x 72mm long x 3mm thick [18] using Instroom 1195 (England). The blade advanced 10mm/ min and the pick up strength of the measuring head was 50kg with the muscle fibers parallel to the direction of the blade. The results were expressed as kg force (f) to shear.

Instrumental Color: The color determination was directly applied on the surface of breast muscle of control and experimental samples after slaughtering and at the 6th day till signs of spoilage begin to appear. A micro color unit attached to a data station (Branolang-Germany) using the standard Cielab color as follows: a-value (redness /green), b-value (yellowness/ blue) and L-value (lightness/ darkness) was used. Six readings were taken at various points on each sample [19].

Bacteriological Examination: Techniques recommended by APHA [20] were used for: Aerobic Plate Count Agar (*Difco Co., Ltd.*) for aerobic plate count; Baird-Parker Agar (*Oxoid, CM 275*) with Egg Yolk-Tellurite Emulsion (*Oxoid, SR 54*) for *S. aureus* count; Lauryl Sulphate Tryptose broth (Oxoid; CM 451) for Coliforms (MPN); Campylobacter selective agar (Oxoid; CM 689) supplemented with 5% lysed horse blood and Campylobacter selective supplement (Oxoid; SR 117) with selective enrichment on Campylobacter enrichment broth (Oxoid; CM 67) with Campylobacter growth supplement (Oxoid; SR 84) and Skirrow supplement (Oxoid; sr 69) for Campylobacter; Xylose Lysine Desoxycholate agar (XLD; Oxoid; CM 469) with enrichment on Rappaport's Vassilidis for Salmonellae; Yersinia selective Agar Base (Oxoid; CM 653) to which Yersinia selective supplement (Oxoid; SR 109) was added and enrichment on modified Rappaport broth for Yersinia enterocolitica.

Statistical Analysis: The results are presented as the mean of three replicates with standard division. Analytical test used unpaired student t-test for comparing means of each treated group independently with control [21].

RESULTS AND DISCUSSION

The data obtained in Table 1 illustrated that the mean pH of chicken meat in group 1 (control) and group 2 (chicken fed on *Bacillus subtilis* mixed with diet) and group 3 (chicken fed on diet mixed with zinc bacitracin) were 6.00, 5.76 and 5.79 at 24 hours after slaughtering (0 time), respectively. It reached to 6.80 when signs of spoilage appeared at 4th day in the control group.Moreover, it increased to 6.50 and 6.72 for group 2 and group 3, respectively at 8th days of chilling at 5°C. pH was significantly reduced (P<0.05) in both carcasses of treated chickens with probiotics than control ones. Similar results were obtained by Pelicano *et al.* [22]. However, Forrest *et al.* [23], stated that muscle transforms into meat due to some biochemical processes, among them alterations in pH which is close to 7.4 *in vivo*. Moreover, Sanudo [24] confirmed that meat quality is influenced by alterations that occur on the pH during rigor mortis. Water holding capacity, cooking loss and shear force are closely correlated to each other in the process of meat tenderness, which is a determinant qualitative factor and one of the most important sensory characteristics of meat [25]. The increase of pH of examined samples were closely related to each of Water holding capacity and TBA values as well as there was a reversible relation with cooking losses (Tables 3 and 4). W.H.C was significantly higher in treated groups than control ($p < 0.05$) as shown in Table 1. This agrees with that reported by Pelicano *et al.* [22]. TBA were significantly decreased (P<0.05) in treated chicken with probiotics than control. It is interesting to note that water loss reduces the meat nutritional value because some nutrients may be lost in the exudates, resulting in a meat less tender and worst in flavor, which was not the case observed in this study. Either of cooking loss and shear force were significantly decreased ($P < 0.05$) in groups 2 and 3 which fed on probiotics.

Table 1: Mean values of pH, water holding capacity and TBA of breast muscle of broilers (No. = 30) fed on diet supplemented with Probiotics

			Experimental groups	
Parameter	Time/day	Group 1 (Control)	Group 2	Group 3
pH	0 time	6.00 ± 0.05	5.76 ± 0.04*	5.79 ± 0.02*
	2 days	6.21 ± 0.03	5.80 ± 0.02*	5.82 ± 0.02*
	4 days	6.80 ± 0.05	5.90 ± 0.05*	5.94± 0.03*
	6 days	7.20 ± 0.06	6.25 ±0.03*	6.28± 0.02*
	8days	ND	6.50 ± 0.04*	6.72 ± 0.02*
Water holding capacity %	0 time	49.21 ± 1.41	52.12 ± 1.05*	52.62±1.43*
	2 days	49.65 ± 1.32	52.34 ± 1.45*	52.75±1.02*
	4 days	50.33± 1.50	52.42 ± 1.04*	52.82±1.23*
	6 days	50.52 ± 1.22	53.26 ± 1.50*	53.97±1.40*
	8days	ND	53.55 ± 1.23*	53.20±1.35*
TBA	0 time	0.33 ± 0.03	0.20 ± 0.05	0.23 ± 0.07
	2 days	0.43 ± 0.03	0.25 ± 0.05*	0.28 ± 0.07*
	4 days	0.89 ± 0.03	0.32 ± 0.02*	0.36 ± 0.09*
	6 days	0.99 ± 0.03	0.43 ± 0.03*	0.43 ± 0.03*
	8days	ND	0.56 ± 0.04*	0.58 ± 0.08*

* Mean significantly differ at P < 0.05. T-test was done between each treated group and control separately.

Table 2: Mean values of cooking loss and texture (shear force/kgf) for Broilers (No. = 30) fed on diet supplemented with Probiotics

Parameter	Time/day	Group1(Control)	Group 2	Group 3
Cooking loss	0 time	21.28 ± 0.21	19.40 ± 0.32*	19.23± 0.01*
	2 days	20.92 ± 0.33	18.92 ± 0.25*	18.20± 0.24*
	4 days	20.58 ± 0.25	18.59 ± 0.13*	17.41±0.45*
	6 days	17. 34 ± 0.51	18.12 ± 0.20*	17.25±0.25*
	8days	ND	17.20 ± 0.50*	17.80±0.62*
Texture	0 time	9.9 ± 0.55	9.9 ± 0.33	10.72 ± 0.23
	2 days	6.8 ± 0.62	9.78 ± 0.24*	10.57±0.35*
	4 days	5.7 ± 0.31	9.62 ± 0.20*	10.20±0.25*
	6 days	5.3 ± 0.50	6.40± 0.22*	7.37 ± 0.31*
	8days	ND	6.00 ± 0.80*	6.42 ± 0.52*

* Mean significantly differ at P < 0.05

ND= non detectable

Table 3: Mean values for color of breast samples (No. = 30) fed on Diet supplemented with Probiotics

	Time / day	Group1(Control)	Group2	Group3
0 time	L (lightness)	48.38A± 0.22	52.67A± 0.32*	51.82A± 0.93*
	a (redness)	4.53B± 0.02	3.02B± 0.10*	3.31B± 0.03*
	b (yellowness)	4.20C± 0.53	5.86C± 0.55*	5.95 C± 0.33*
6 day	L(lightness)	45.42a± 0.21	51.28a± 0.5*	50.75a± 0.89*
	a (redness)	5.60b± 0.05	3.56b± 0. 3	4.20b ±0.55
	b (yellowness)	3.38c± 0.56	4.95c± 0.26*	4.02c± 0.5*

* Mean significantly differ at P < 0.05. Presence of a capital letter and small letter for each independent in each column indicate significantly differ by-T-test which was done also between each parameter at 0-time and the same parameter after 6th day

Table 4: Statistical analytical results of Aerobic plate count at 35 C, *S. aureu count* and Coliforms MPN of Broilers (No. = 30) fed on diet supplemented with Probiotics

	Group 1 (Control)	Group 2	Group 3
	Means counts + S.E. ($\log_{10}$ cfu/g)		
Aerobic plate count at 35 °C	6.35 ± 0.72	4.81 ± 0.25*	3.50 ± 0.54*
S. aureus count	4.21 ± 0.28	2.18 ± 0.15*	2.24 ± 0.20*
Coliforms MPN	3.50 ± 0.20	2.40 ± 0.20*	2.92 ± 0.25*

*Mean significantly differ at (P < 0.05)

Table 5: Incidence of some pathogens in broilers (No. = 30) fed on diet supplemented with Probiotics

	Group 1 (Control)		Group 2		Group 3	
	No	%	No	%	No	%
C. jejuni	10 A	33.3	6 a	20	*ND a	ND
Salmonellae	5 B	16.6	ND b	ND	ND b	ND
Y. enterocolitica	11C	36.6	7.0 c	23.3	4.0 c	13.3
E. coli	12 D	40	10 D	33.3	9.0D	30

*ND = non detectable

Small letter in the same row means significantly differ (decrease) at P < 0.05 than control.

In contrary, Pelicano *et al.* [22] recorded that water holding capacity and cooking loss were not different among probiotics and control. The tenderness was appeared after two days in control samples (6.8 ± 0.62) as well as it was (6.40 ± 0.22) and (7.37 ± 0.31) in treated samples (chicken fed on diet mixed with bacillus subtilis and chicken fed on diet mixed with zinc bacitracin) respectively after 6th days (Table 2). This agrees with that reported by Lyon and Lyon [26] who considered that values up to 7.5 kgf/g might be consider as tender, while Simpson and Goodwin [27] proposed values up to 8kgf/g. On the other hand, Contreras [28] reported that shear force values in breast muscle were between 5.5 to 5.8kgf/g. considering these reference values, probiotics didn't affect meat tenderness, since SF values were 6.00 ± 0.80 and 6.42 ± 0.52 till the 8th day of storage in refrigerator.

The data obtained in Table 3, showed that color L-*(lightness) and b-* (yellowness) values were significantly increased in carcasses of chicken fed on diet mixed with *Bacillus subtilis* and zinc bacitracin than control. Redness (a-* value) was significantly increased in the control group than treated groups with probiotics. The present data revealed that L-* (lightness) and b-*(yellowness) were significantly decreased at the 6th day during refrigerator storage at 5°C in control group while in treated groups (2 and 3) there were no significant differences. The (a-* value redness) was significantly increased in control and in treated groups with probiotics; there were no significant differences. In this respect, Northcutt [29] stated that, meat color alterations, which occur in swine such as PSE (pale soft and exudative) and DFD (dark firm and dry) are rare in birds. Nevertheless, changes in color that are similar to PSE have already been described in broilers. One of the most important methods to identify such alterations in meat are objective colorimetric measurements from the CIELAB system which determines the parameters L*,a* and b* [30]. pH of control groups was gradually increased and this accompanied by darker meat, L* and b* values were lower than treated samples. While a* values were higher in control than treated groups. However, this finding was in accordance with previous reports [31,32]. In this respect, Allen *et al*. [15] reported that a significant correlations existed between pH and color as well as odor. The darker chicken breast meat has a shorter shelf-life than lighter breast meat. The shorter shelf-life may be due to differences in pH [30, 31,33]. From the present data, it could be concluded that the increase in pH was accompanied by increase in water holding capacity and decrease in cooking loss., as well as darker meat than normal and tender meat. The incorporation of probiotics as feed supplement in broiler diet keeps the pH of meat in normal range for long time. The extension of shelf-life time during preservation of treated groups leads to increase of tenderness of meat. Probiotics improve the pH, water holding capacity, TBA, Cooking loss, Shear-force, color of chicken meat and extend its shelf life time. The data obtained in Table 4 revealed that the Aerobic plate, *S. aureus* and Coliforms (MPN) were significantly decreased ($P < 0.05$) in chicken fed on diet supplemented with probiotics as compared with control. The bactericidal effect of probiotics was probably accompanied by production of antibodies. This confirmed the hypothesis reported by Chaveerach *et al*. [34]. The reduction of microbial load may be due to production of different antimicrobial components by probiotics such as organic acids, hydrogen peroxides, carbon peroxides, diacetyl, low molecular weight antimicrobial substances, bacteriocins and adhesion inhibitors. Some bacteriocins produced by specific probiotics strains can fulfill a role in the inhibition of common broiler pathogens. This agrees with previous reports [35,36]. However, Probiotics exhibited antimicrobial activity against a broad spectrum of bacteria found in the broiler gastrointestinal tract including food spoilage and pathogenic organisms such as *Bacillius species*, *Cl perfringenes, S. aureus* and *listeria monocytogenes*. This held the view reported by Barbosa *et al*. [37].

The data obtained in Table 5 showed that the incidences of isolation of *C. jejuni, Salmonellae, Yersinia enterocolitica* and *E. coli* were significantly decrease ($p < 0.05$) in chickens fed on diet supplemented with probiotics in compared with the control. On the other hand *E. coli* was not significantly reduced in groups fed on diet supplemented with probiotics than the control. Intestinal colonization of *C. jejuni* in the chickens plays a role in contamination during slaughter. Thus reducing *C. jejuni* colonization in chickens can potentially reduce the incidence of C. jejuni infections in human [38]. The administration of antibiotics did not significantly reduce campylobacter shedding, *C jejuni* exclusively was found during both rearing and on the carcasses. A significant correlation exits between the contamination of the broilers during rearing and the carcass after processing. [38]. Similar results were obtained by Fritts *et al*. [39] who add *B. subtilis* from 1 to 42 days of rearing of birds. *B subtilis* cause reduction in APC, Coliforms (non *E. coli*) and Campylobacter. For Salmonellae, 94 carcasses of birds fed control diet were positive for salmonella, 41 of 96 of carcasses of bird fed probiotics were positive also.

It could be concluded that supplementation of chicken diet with probiotics improves the quality of chicken carcasses and also reduce its microbial load.

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