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Effect of dietary probiotics on the growth performance, meat quality improvement of broiler chicken for safe meat production

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Abstract: A feeding trial was conducted to investigate the effect of Probiotic (Biofast) on the growth performance and meat quality of broiler chicken. Ninety six 1-d-old mix sexed Cobb 500 broiler chicks were obtained from a local commercial hatchery. Chicks were randomly allocated in 2 experimental treatments for 5 wk. The experimental treatments received a 1) control, 2) control with probiotic Biofast 0.055% (*bacillus subtilis*-100%) in the diet. Both treatments had 48 broilers arranged in 4 replicates of 12 broilers each. Eight birds from 2 different groups (one/replication) were sacrificed on termination of the feeding trial of breast, thigh to investigate the meat quality. Feed intake in treatment T₁ was significantly ($P > 0.05$) greater than control in 0 to 3 weeks of age. Though in starter phase treatments failed to induce any marked effects on body weight, weight gain and FCR but numerically increased in T₁ than control. In finisher period (4-5 wks), there were no significant differences on body weight, weight gain, feed intake and FCR between the treatments. The organ weight like liver, heart, kidney, spleen, gizzard, abdominal fat and intestine weight also did not show any significant differences between the dietary treatments of control and Biofast. In addition, though the dressing % was not significantly different but numerically higher percentage was found by using Biofast. Similarly, no significant differences ($p > 0.05$) were found in pH, cooking loss, meat color and TBA values between the treatments. In conclusion, supplementation of Biofast in diet has no significant effect on the growth performance of broiler chicks though it has got some positive effects on other parameters that indirectly revealed to enhance meat quality of broiler chicken as well as food safety issues.

Keywords: broiler chicken; probiotics; growth performance; meat quality; safe meat

1. Introduction

There is an increasing demand for quality in animal products, as well as concern about the effect of these products on human health. For this reason, animal production systems will have to focus not only on obtaining high production, but also on their impact on the environment as well as on human and animal health (Ferket, 2003). Consumer has also been increasingly accepting alternative therapies which include probiotics, in replacing synthetic drugs. Moreover, their use in the poultry industry has increased as potential alternatives to antibiotics use as growth promoter, and in select cases, for controlling specific enteric pathogens (Ezema, 2013). Probiotics are feed additives that contain live microorganisms and promote beneficial effects on the host of favoring the balance of the intestinal microbes (Fuller, 1989). In many countries of the world, including Bangladesh the use of most antibiotics growth promoter (AGP) has been banned to preserve the effectiveness of

important human drugs (Casewell, 2003). Recently, alternatives for substituting these traditional growth promoters have been evaluated and probiotics feeding have been the area of interest. The probiotics include live bacteria, yeast, their metabolites and pH adjusters, which contribute to maintain balance in intestinal microflora (Islam *et al.*, 2004). Therefore, probiotics has been used as natural biological non-feed additives which have beneficial effects to poultry by improving its intestinal microbial balance to stimulate the processes of digestion and absorption of nutrients (Pelicano *et al.*, 2002). Probiotic microorganisms are responsible for the production of vitamin B complex and digestive enzymes for stimulation of intestinal immunity, increasing protection against toxins produced by pathogenic organisms. In broiler nutrition, probiotic species such as *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* are widely used to prevent poultry pathogens and diseases and improve broiler's growth performance (Zulkifli *et al.*, 2000; Kabir *et al.*, 2004). Probiotic act as a mono or mixed culture of living microorganisms which beneficially affect the host by improving the properties of the indigenous microflora. The use of *Bacillus subtilis* spores as a probiotic or a direct-fed microorganism could be an alternative to adding to feed for better growth performances and immunity of broiler. In Bangladesh, most of the feed company export probiotic from foreign countries. Since there have been a few investigations on the effects of *Bacillus subtilis* in broiler feed. Recently, Biofast which is 100% *Bacillus subtilis* could be used to supplement with feed for better growth performances and immunity of broiler. Meanwhile, the challenge for nutritionists will be to obtain well-balanced cost effective feed which will be free from antibiotics and ensure the safety of poultry products for consumers. These factors may be critical in the Bangladesh, where most of the farmers have been used antibiotics into their poultry feed to enhance birds performance and disease resistant capacity. The poultry feed industry needs adequate information on this aspect to augment commercial broiler production in Bangladesh. Furthermore, there is a way to increase the use of probiotics in diets for animals, which is a more reasonable option, since they do not leave residues in the environment, in the animal body and do not cause cross-resistance in men compared with antibiotics (Nepomuceno and Andreatti, 2000). In this context, this study was undertaken to evaluate the effect of probiotic (Biofast powder) which is supplemented in diets on performance and carcass characteristics of broilers from 1 to 35 days.

2. Materials and Methods

2.1. Birds and housing

Ninety six 1-d-old mix sexed Cobb broilers were obtained from a local commercial hatchery. Broilers were randomly allocated in 2 experimental treatments for 5 wk. Each treatment had 48 broilers arranged in 4 replicates of 12 broilers each. Each replicate was assigned to a clean floor pen (2 m²) and birds were raised on a rice straw based litter. Heat was provided with a heating lamp per pen. The ambient temperature in experimental house was maintained at 32°C during the first week and thereafter decreased by 3°C in the third week, and finally fixed at 22°C up to end of the experiment. The experiment lasted for 35 day.

2.2. Dietary treatments

To meet the nutrient requirements of the broiler chicken over this period, a complete basal diet was formulated for each of the 2 stages of growth; starter and finisher. The diets were formulated to meet the nutrients requirements of broilers as recommended by the National Research Council (NRC, 1994). The experimental treatments received a 1) control, 2) control with probiotic Biofast 0.055% (*Bacillus subtilis*-100%). Biofast was prepared according to manufacturer instructions (4500g rice polish should be mixed with 250 g Biofast powder. 1 kg from that mixture needed for the preparation of 100 kg poultry feed). The basal diet was formulated for starter (1 to 21 d) and finisher (22 to 35 d) of broiler growth periods and its composition is shown in Table 1. The basal diet was prepared in each week and stored in sacks and was kept in a cool place. Experimental diets and water were provided *ad libitum*.

2.3. Growth performance traits

Growth performance parameters such as body weight (BW), weight gain (WG), feed intake (FI), and feed conversion ratio (FCR), were determined every week. Starter (0-3 wks) and finisher (4-5wks) BW gain, FI, and FCR were calculated for the whole duration of the experiment.

2.4. Organ weights and carcass yield percentages

At the end of experiment, after weighing, 4 birds per treatment were randomly selected and killed by cervical dislocation. The liver, heart, kidney, spleen gizzard, abdominal fat and intestine were excised and weighed. Afterward, the birds were scalded, defeathered, and carcasses were eviscerated. The head and feet were

removed, and calculated as a percentage of live body weight and also carefully examined to detect any pathological lesion or damages. The weight of intestine was also measured and recorded.

2.5. Meat characteristics

Muscular pH values were determined on Pectorals major muscle with a needle probe 24h post mortem with Mettler MP 120-B digital pH-meter. All pH measurements were conducted on the anterior end of the right breast. The pH meter was standardized by a two-point method against standard buffers of pH 4.0 and pH 7.0. Cooking loss was analyzed as [sample weight before cooking minus sample weight after cooking] $\times 100$ / sample weight before cooking. The colour of breast meat was determined after 24 h of cooling the carcass with Minolta CR-400 colorimeter (MINOLTA CAMERA Co. Ltd., Osaka, Japan) calibrated against white plate (CIE L^* – lightness, a^* – redness, b^* – yellowness) with 8 mm optical probe diameter, D65 illuminant and 2° observer. The meat colour is presented as CIE- $L^*a^*b^*$ (Commission Internationale de l’Eclairage, 1976). Depending on the colour breast meat samples were classified into following groups: DFD ($L^* < L^*_{44}$) – Soares *et al.* (2002, 2009).

Meat samples (5 g) from each breast cut were used for the analysis of the thiobarbituric acid reactive substances (TBARS) by using the aqueous acid extraction method of Pikul *et al.* (1989) to determine lipid oxidation. The reaction produces a red color which can be measured using a spectrophotometer.

2.6. Statistical analysis

All data were subjected to analysis of variance procedures appropriate for a completely randomized design using the general linear model procedures of SAS (SAS Inst. Inc., Cary, NC 2005). The mean differences among different treatments were separated by Duncan’s multiple range tests. A level of ($P < 0.05$) was used as the criterion for statistical significance.

3. Results and Discussion

This study indicated that feed intake in treatment T_1 was significantly ($P > 0.05$) greater than control in 0 to 3 weeks of age (Table 2). Though in starter phase treatments failed to induce any marked effects on body weight, weight gain and FCR but numerically increased in T_1 than control. In Table 3, there were no significant differences on body weight, weight gain, feed intake and FCR between the treatments (T_0 , T_1) during the finisher phase in this trial. A significant difference in feed intake between the treatments was observed in the period of 0-21 days of age. In agreement with the present results, Boratto *et al.* (2004) reported that there were no significant differences in weight gain of chicken given diets with or without *Bacillus subtilis* in the diet. Another group of researcher reported that the inoculation of probiotics has no effect on weight gains (Rocha *et al.*, 2010; Takahashi *et al.* 2005) and feed consumption (Cavit, 2004; Yalcinkayal *et al.*, 2008) but reduce feed intake which was verified by Zulkifli *et al.* (2000).

The obtained data showed that there no significant different in body weight with control groups during study. But Probiotic showed numerically the higher body weight than that of control. These results suggested that probiotics used as a feed supplement in diet of poultry to enhance productive performance and immune responses (Higgins *et al.*, 2008). In this regard the dietary supplementation of probiotic have beneficial effect on the host animal by stimulating appetite (Nahashon *et al.*, 1992), stimulate the immune system (Koenen *et al.*, 2004), produce the endogenous digestive enzymes (Saarela *et al.*, 2000), decrease pH and release bacteriocins (Rolfe, 2000). According to Ramarao *et al.* (2004) it was also not possible to observe any influence of probiotics on broiler weight gain, as opposed to Kabir *et al.* (2004), who obtained higher weight gain in broilers fed a probiotic product. Our results also did not find any differences between two groups due to the variation of microbial culture used in the probiotics, application level, feed composition, age and strain of the bird. The parameter feed conversion ratio was not statistically different between treatments in none of the studied intervals, but in starter phase numerically lower in probiotic than control, as also observed in the experiment of Loddi *et al.* (2000), who worked with a probiotic product containing *Enterococcus faecium* (1×10^{10} CFU/g product), other authors, however, obtained better feed conversion ratio in broilers fed probiotics in the periods of 0-21 days (Zulkifli *et al.*, 2000; Maiorka *et al.*, 2001; Corrêa *et al.*, 2003; Pelicano *et al.*, 2004a) and 0-40 days (Maiorka *et al.*, 2001; Boratto *et al.*, 2004). But in finisher phase we found no difference with control group.

The organ weight like liver, heart, kidney, spleen, gizzard, abdominal fat and intestine weight did not show any significant differences between the dietary treatments of Biofast and control. But, abdominal fat was numerically lower than control. Boratto *et al.* (2004) found increased liver size of poultry reared in environment inoculated with bacteria, which may be related to the neutralization of toxic substances produced from the

metabolic activity of intestinal bacteria, which requires a constant energy expenditure made by the liver for detoxification inducing the hypertrophy of hepatocytes. He found the total amount of edible offal and gizzard was higher for poultry fed diets supplemented with probiotics. But our results were opposed to his findings. In addition, though the dressing % was not significantly different but numerically higher number found on T₁ (Biofast) treatment though Boratto *et al.* (2004) found carcass yield percentages were higher for the nonprobiotic-fed femalebroilers than for the control. This points to the fact that probiotic causes suppression in production and processing performance traits.

Table 1-1. Composition of basal diet for the broiler starter and finisher diet.

Item	Starter (1-21d)	Finisher (22-35 d)
Ingredient (%)		
Maize	52	60
Protein concentrate	6	7.4
Rice polish	4.6	3
Soybean	33.3	25.6
Di calcium phosphate	1	1
Vitamin-mineral premix	0.25	0.25
Salt	0.5	0.5
Oil	1.9	2
Lysine	0.1	0.1
Methionine	0.1	0.1
Limestone	0.5	0.3
Calculated analysis (per kg of diet)		
Moisture (%)	10.64	13.47
Ash	7.16	6.06
CP(g)	22.69	16.49
Fat	3.44	7.22
Fiber	4.31	4.62

Table 1-2. Proximate analysis of broiler breast and thigh meat.

Treatment	Meat	Proximate analysis (% on fresh basis)				
		Moisture	CP	EE	CF	Ash
Control	Breast	68.29	23.06	4.87	0.60	1.86
Biofast	Breast	65.79	23.04	3.46	0.50	1.98
Control	Thigh	73.69	20.22	3.24	0.70	1.72
Biofast	Thigh	69.70	19.30	2.41	0.75	1.64

Table 2. Broiler growth performance on starter phase (0-3 weeks).

Treatment	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR
T ₀	761.54	716.54	849.40 ^b	1.185
T ₁	766.32	721.32	857.18 ^a	1.187
SEM	3.705	3.812	1.793	0.007
P-Value	0.56	0.57	0.012	0.87

* T₀ = control, T₁ = Biofast

Table 3. Broiler growth performance on finisher phase (4-5 weeks).

Treatment	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR
T ₀	1818.54	1102.21	2100.15	1.9
T ₁	1836.08	1101.72	2099.72	1.9
SEM	11.167	2.254	15.296	0.014
P-Value	0.47	0.92	0.99	0.93

* T₀ = control, T₁ = Biofast

Table 4. Effects of dietary treatments on absolute organ weights (g) of broiler chickens.

Treatment	Liver	Heart	Kidney	Spleen	Gizzard	Abdominal fat	Intestine	Dressing %
T ₀	42.00	11.75	4.75	2.75	56.25	19.50	144.75	71.58
T ₁	40.50	11.75	4.75	2.75	52.50	17.25	140.00	72.40
SEM	0.548	0.411	0.453	0.25	1.266	1.164	1.889	0.264
P-Value	0.11	1.00	1.00	1.00	0.14	0.37	0.23	0.12

Table 5. Different meat quality characteristics of broiler feeding with dietary Probiotic (Biofast).

Treatment	p ^H	Cooking loss	Color values		
			L*	a*	b*
T ₀	6.30	18.36	51.23	3.48	9.01
T ₁	6.23	18.25	53.33	4.44	8.21
SEM	0.054	0.814	0.724	0.406	0.232
P-Value	0.56	0.95	0.16	0.26	0.07

* Meat color values of lightness (L*), redness (a*), and yellowness (b*)

Table 6. Thiobarbituric acid (TBA) value of broiler feeding with Biofast.

Treatment	TBA Value
T ₀	8.81
T ₁	8.63
SEM	0.566
P-Value	0.344

Table 7. European Production Efficiency Factor (European Broiler Index).

Item	Dietary treatment	
	Control (n=48)	Probiotic (Biofast) (n=48)
FCR (0-5wks)	1.84	1.83
Mortality (0-5wks)	2.08	2.08
Percentage	276.42	280.68
*EPEF		

*EPEF = European Poultry Efficiency Factor (Average gram gained/day × % Survival rate /FCR × 10).

No significant differences ($p > 0.05$) were found in pH, cooking loss and meat color between the treatments as shown in Table 5. According to Ludtke (2009), due to the rapid metabolic transformation of glycogen into lactic acid, which results in achieving ultimate pH before carcass cools, causing protein denaturation, and consequently, meat becomes pale, soft, and exudative. In probiotic, pH value was slightly lower than control. In control, lightness of meat was numerically higher than probiotic. L* value is the main parameter that determines poultry meat color. Color is one of the main indicators of the quality of most foods. This sensorial quality has a high influence of the meat purchase decision and its acceptance by consumers. It is an important functional quality and it is closely related to other qualities, such as pH, water holding capacity, emulsifying capacity, and texture. Whereas tenderness is one of the main sensorial attributes that determine global acceptability, meat color is associated to acceptability at purchase (Bressan and Beraquet, 2002; Sanders *et al.*, 1997). In Table 6 represented that thiobarbituric acid (TBA) values were not significantly different between the treatments. According to Gheisari (2011), the extent of oxidative rancidity in a fat may also be determined by its TBA number. The 2- thiobarbituric acid (TBA) test is believed to measure the breakdown products of unsaturated fatty acid oxidation. Typically, the TBA number of a sample shows a steady increase as it becomes more rancid, but a certain amount of variation is found between the TBA numbers obtained for similar fresh samples. In this study the Biofast showed numerically decreased number than the control. The mortality percentage and the European production efficiency factor are presented in Table 7. The mortality rate was same (2.08%) for both the group (2.08%). The European production efficiency factor was greater for the Biofast-supplemented group (280.68) than control group (276.42).

4. Conclusions

The addition of the probiotic product, Biofast to broiler diets didn't show significant influence on the performance compared to those of control but showed numerically higher body weight, dressing percentage and higher European Broiler Index and at the same time lowered abdominal fat and cooking losses. It can be concluded that probiotic has some positive effects on the parameters that indirectly revealed to enhance meat quality of broiler chicken as well as food safety issues. Further follow-up study is necessary to determine Biofast inoculation levels in the broiler diet.

Conflict of interest

None to declare.

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