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Synergistic Effect of Supplementation of Coated Sodium Butyrate and *Bacillus Subtilis* on Cost Effectiveness in Broiler Chicken Production

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ABSTRACT

The present experiment was carried out to assess the synergistic efficiency of supplementation of coated sodium butyrate and *Bacillus subtilis* as a substitute for antibiotic growth promoter in broiler diet and to work out the cost effectiveness. A biological trial was carried out with 216 day–old cobb 400 broiler chicks distributed to six experimental groups with six replicates, each replicate containing six chicks. The experimental groups were fed with basal diet (T1), a basal diet with antibiotic (T2), probiotic (*Bacillus subtilis*) alone (100 g/ton of diet) (T3), and coated sodium butyrate (lipid encapsulated) high at 0.18 (T4) or medium 0.09 (T5) and low 0.045 (T6) per cent levels combined with *Bacillus subtilis (*1x 1012 cfu/g*)* (100 g/ton of diet). The trial was conducted in a deep litter pen and last for 35 days. The increase in the revenue of Rs. 4.31 and Rs. 1.61 per kg live weight gain in the group supplemented with CSB at 0.09 per cent and *Bacillus subtilis* 100 g/ton of diet (T5) as compared to basal diet and antibiotic group (T2), respectively. In conclusion, it is recommended to include the CSB at 0.09 per cent and *Bacillus subtilis at* 100 g/ton of feed as an alternate for antibiotics in the broiler diet.

KEY WORDS: *Bacillus subtilis*,Broiler, Coated sodium butyrate, Cost effectiveness

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INTRODUCTION

In modern commercial production, the broiler chickens are exposed to physiological stress resulting in diminishing immune competence and further affects gut health. To overcome these losses, several antibiotics have been routinely used in poultry diets to prevent disease and to improve performance. The regular practice of using antibiotic growth promoters had possibly led to the development of antimicrobial resistance. Usage of antibiotic growth promoters (AGPs) has been banned in European Union since January 2006 (Griggs and Jacob, 2005). Prohibited use of antibiotics as growth promoters in poultry feed leads to an increase in the interest to find alternate microbial load suppressing agents (FAO, 2016). Probiotics in poultry help in maintaining normal intestinal microflora by competitive exclusion antagonism, lowering the pH through acid fermentation, competing for mucosal attachment and nutrients, producing bacteriocins, stimulating the immune system associated with the gut and increasing the production of short chain fatty acids (Sarangi et al., 2016). Organic acids can penetrate a bacterial cell wall and disrupt its regular physiology as well as reducing the pH of the digesta, increase pancreatic secretions and can also have trophic effects on the mucosa of the gastrointestinal tract of animals (Adil et al., 2010). Short chain fatty acids are preferred acidifiers, among which, butyric acid is considered as the major energy source for enterocytes, essential for the development of Gut Associated Lymphoid Tissue (GALT) (Friedman and Bar-Shira, 2005) and has the maximum bactericidal efficacy against the acid intolerant species such as *Escherichia coli* and *Salmonella* spp. (Kwan and Ricke, 2005). Blend of organic acids and probiotics was attributed forbetter intestinal morphology and microbial ecosystem when compared with the single use of either organic acids or probiotics (Rodjan et al., 2018). Probiotic *Bacillus subtilis* was chosen for this study because it can form endospores under unfavourable conditions (Ulrich et al., 2018). Based on the above background, the present experiment was undertaken to assess the synergistic response of coated sodium butyrate (CSB) and in combination with probiotic (*Bacillus subtilis*) as a substitute for antibiotic (oxytetracycline) on cost effectiveness of broiler chicken production.

MATERIALS AND METHODS

Experimental details and data

The biological experiment was carried out at a deep litter pen in the Department of Animal Nutrition, VeterinaryCollege and Research Institute, Namakkal, Tamil Nadu from March to April 2021 for 35 days. The biological trial was carried out with 216 day–old Cobb 400 broiler straight run chicks distributed to six experimental groups with six replicates, each replicate containing six chicks. The experimental groups were fed with basal diet (T1), a basal diet with antibiotic (T2), probiotic (*Bacillus subtilis*) alone (100 g/ton of diet) (T3), and coated sodium butyrate high at 0.18 (T4) or medium 0.09 (T5) and low 0.045 (T6)per cent levels combined with *Bacillus subtilis* (100 g/ton of diet).

The antibiotic used in this study was 20 % oxytetracycline (TM-200) at the rate of 250 mg/kg of feed (concentration 50 ppm). *Bacillus subtilis* probiotic supplement (PB6- strain) was procured from M/s. Kemin Industries South Asia Pvt. Ltd., Chennai, India (CLOSTAT™ 12 Dry Kemin consists of 1 x 10^{12}cfu/g). The concentration and viability of *Bacillus subtilis* colonies were evaluated at Poultry Disease Diagnostic Surveillance Laboratory, Namakkal and stored at room temperature. The coated sodium butyrate (M/s. Excentials Butycoat, Orffa Excentials, Netherland and marketed by Orffa Animal Nutrition Pvt. Ltd., Maharashtra, India) was encapsulated with vegetable fatty acid containing 30 per cent sodium butyrate. The level of coated sodium butyrate (CSB) was increased by three times since the active compound (SB) in it was 30 per cent (as per the analysis of test sample). The pre-starter, starter and finisher diets were formulated by using iso-energy and iso-nitrogenous nature with aflatoxin-free ingredients to meet the ileal amino acid digestibility, metabolizable energy and other nutrients requirements as per the breeder specification. The ingredient composition of the experimental ration of broiler pre-starter, starter, and finisher diet are presented in Table 1.

Table1. Ingredients and nutrient composition of experimental broiler pre-starter, starter and finisher diets

Ingredient	Pre starter diet	Starter diet	Finisher diet
Maize (%)	53.8	59.3	61.6
Soybean meal (%) CP – 42%	39.8	33.6	29.6
Rice bran oil (%)	2.38	3.50	5.21
Calcite (%)	1.25	1.34	1.19
Dicalcium phosphate (%)	1.12	0.98	1.07
Sodium chloride (%)	0.29	0.29	0.29
L-Lysine hydrochloride (78 %) (kg/100kg)	0.19	0.18	0.17
DL - Methionine (99 %) (kg/100kg)	0.29	0.25	0.23
L - Threonine (kg/100kg)	0	0	0
Additives and supplements* (g/100kg)	470	470	470
NSP enzyme1 (kg/100kg)	0.05	0.05	0.05
Choline chloride[2] (kg/100kg)	0.100	0.10	0.10
Soda bicarbonate [3] (kg/100kg)	0.207	0.25	0.20
Total	100.066	100.318	100.268

Ingredient	Pre starter diet	Starter diet	Finisher diet
Nutrients**			
Crude protein (%)	23.4	21.0	19.5
Digestible lysine (%)	1.25	1.10	1.00
Digestible methionine (%)	0.60	0.53	0.49
Metabolisable Energy (kcal/kg)	3000	3125	3250
Calcium (%)	0.94	0.92	0.88
Available phosphorus (%)	0.49	0.45	0.46

**Calculated value

*Additives added 470 g/100kg containing Ultra-TM – 100g, Coxistac – 50g, Adphos5.0 – 10g, Zigbir – 100g, Rovimix – 50g, Vit C-50g, Sele-H – 10g, US-Curatox – 100g

1. Enzymes: Each kg containing Cellulase-1,20,00,000; Hemicellulase-54,00,000; Protease - 24,00,000; Amylase - 24,00000; Beta glucanase -1,06,000 IU (NSP enzyme)
2. Choline chloride -100g /100kg
3. Soda bicarbonate – 200 g/100kg
4. Trace mineral mixture - Each 5kg of contains Manganese:270gm, Zinc:260gm, Iron:100gm, Iodine:10gm, Copper:10gm, Selenium;1.5gm (Ultra-TM premixture)
5. Salinomycin Sodium 12% Composition: A standardized formulation of Salinomycin sodium12% w/w premix on a specially selected carrier (Coxistac-120)
6. MicrogranulatedVelocious Phytase Ingredients: Comprises 5000 IU/gram of phytase enzyme (Adphos-5.0)
7. Liver tonic
8. Vitamin mixture: Each kg contains VitaminA-20,00,000 IU Vitamin D3-6,00,000 IU Vitamin E- 16,000g Vitamin K3- 3,000g, Vitamin B1- 2,000g, Vitamin B2-10,000g, Vitamin B6-3,000g, Vitamin B12- 0.016g, Pantothenic Acid- 16,000g Folic Acid- 0.5g, Niacin- 26,000g . (Rovimix)
9. Vit C – 50g/100kg
10. Antioxidant: Each 250 (g) contains Vit E-20000 mg, selenium – 250 mg, biotin – 80 mg (Sele-H)
11. Toxin binder: Unique multi spectrum toxin control formula (US CuraTox-Fs)

The standard and uniform managemental practices were followed by all the treatment groups throughout the experimental period. The chicks were fed with the weighed quantity of experimental diets and had free access to water.

Cost effectiveness

The economics of raising broilers up to 35 days with different levels of CSB with *Bacillus subtilis* supplementation was calculated based on the actual cost of feed per kg live weight gain.

Statistical analysis

The data collected on various parameters were statistically analysed as per the method of Snedecor and Cochran (1989). One-way analysis of variance (ANOVA) with Tukey's post hoc test was performed to assess the significance of differences among control and experimental groups. The analysis was carried out by SPSS software package (20.0, Inc. Chicago, USA).

RESULTS AND DISCUSSION

Cost effectiveness

The effect of supplementation of antibiotic (T2), probiotic *Bacillus subtilis* alone (T3) with combinations of 0.18 (T4), 0.09 (T5) and 0.045 (T6) per cent CSB on the cost effectiveness in broiler production are presented in Table 2.

Table 2. Effect of coated sodium butyrate and *Bacillus subtilis* supplementation at different levels on cost effectiveness in broiler chicken production

Treatment	T1	T2	T3	T4	T5	T6
Cost of feed(Rs.)/ kg						
Pre-starter	35.3	35.5	35.4	36.4	35.9	35.7
Starter	34.7	34.8	34.8	35.7	35.2	35.0
Finisher	37.4	37.5	37.5	38.5	38.0	37.8
Feed intake (g)/ bird						
Pre-starter	532.4 ±13.82	521.2 ± 7.66	529.3 ± 5.99	547.8 ± 7.77	535.1 ± 7.57	525.4 ± 17.6
Starter	1424.9± 33.7	1432.9 ± 35.9	1426.6± 17.9	1434.9 ± 28.6	1425.9 ± 17.7	1422.2 ± 32.7
Finisher	1167.0 ± 10.49	1058.0 ± 18.71	1067.9 ± 14.35	1087.9 ± 34.55	1037.5 ± 15.7	1082.07 ± 40.1
Cost of feed (Rs.)/ bird						
Pre-starter	18.8 ± 0.48	18.5 ± 0.27	18.7 ± 0.21	19.9 ± 0.28	19.2 ± 0.27	18.7 ± 0.63
Starter	49.4 ± 1.17	49.9 ± 1.25	49.6 ± 0.62	51.2 ± 1.02	50.2 ± 1.53	49.8 ± 1.14
Finisher	43.6 ± 0.39	39.7 ± 0.70	40.1 ± 0.53	41.8 ± 1.32	39.4 ± 0.59	40.8 ± 1.51
Overall feed cost (Rs.)	111.9	108.2	108.5	113.0	108.9	109.41
Cumulative body weight gain (g)/ bird	1892	1917	1954	2032	1989	1969
Mean change in feed cost/kgliveweightgain (Rs.)	59.1 ± 0.75	56.3 ± 0.67	55.57 ± 0.62	55.74 ± 1.55	54.8 ± 0.83	55.68 ± 1.66
Mean change in feed cost/kglive weightgain over antibiotic group (Rs.)	-2.70 ± 0.75 (-4.79%)	0	0.86 ± 0.62 (1.53%)	0.69 ± 1.55 (1.22%)	1.61 ± 0.83 (2.86%)	0.75 ± 1.66 (1.33%)
Mean change in feed cost/kglive weightgain over control group (Rs.)	0	2.70±0.67 (4.57%)	3.56±0.62 (6.02%)	3.39±1.55 (5.73%)	4.31±0.83 (7.29%)	3.45±1.66 (5.83%)
Supplement cost in Rs. for the total feed consumed by the group	0	0.44^{c} ± 0.00	0.31^{b} ± 0.00	3.34^{f} ± 0.21	1.71^{e} ± 0.01	1.02^{d} ± 0.02

Mean ± SEof six observations; Mean feed intake and bodyweightgain ofthe treatmentgroupsare present.

Means having different superscript within a row differ significantly ($P<0.05$) Cost of Bacillus subtilis-Rs.1040/kg; Costof Coated Sodium Butyrate - Rs. 520/kg; Costof oxytetracycline - Rs 615/kg

T1- Control ration without antibiotic; T2 -T1 + antibiotic (OTC @ 50 mg/kg); T3 - T1 + Bacillus subtilis (100 g/ton); T4- T1 + CSB (0.18 % of diet)+ Bacillus subtilis (100 g/ton); T5 - T1 + CSB (0.09 % of diet) + Bacillus subtilis (100 g/ton); T6 - T1 + CSB (0.045 % of diet)+ Bacillus subtilis (100 g/ton)

The actual cost of feed (Rs.) per bird in pre starter, starter and finisher phases (0-35 days) of experimental broiler birds were calculated by using the data of respective cost of feed per kg and feed intake (g/bird) at different treatment groups. The actual cost of feed (Rs.) per bird during pre-starter, starter and finisher phases are clubbed together to get total cost of feed/bird and it is divided by respective cumulative body weight gain of the different treatment groups and obtained feed cost per Kg live weight (Rs.), respectively.

The cost of feed per kg live weight gain was found to be lowest in T5 group (Rs. 54.82), due to its lower feed intake, higher body weight gain and better FCR. Even though the treatment groups supplemented with antibiotic (T2- Rs. 56.34), probiotic alone(T3- Rs. 55.57), or combination with 0.18% CSB (T4- Rs. 55.74), and 0.045% CSB (T6- Rs. 55.68) were found to be slightly higher as that of 0.09% CSB with probiotic supplemented groups (T5- Rs. 54.82). Similarly, Gomathi et al. (2021) reported that supplementing cinnamon oil (at 250 mg/ kg diet) with 0.09 per cent of CSB (Rs. 49.80) had better economical returns over the live weight gain when compared to control (Rs. 52.59) and it was comparable to that of antibiotic supplemented group (Rs. 49.37).

In the present experiment, the basal diet group had the highest (Rs. 59.14) cost of feed per kg live weight gain among the treatment groups (Rs. 54.82 to 56.34). The supplementation of probiotic with a combination of 0.09 per cent CSB (T_5- Rs. 4.31) had better economical returns when compared to the basal diet. Further, it was saved 7.29 per cent of the feed cost over basal diet due to lower feed intake, higher body weight gain with better FCR. Similarly, when compared to control, the birds supplemented with antibiotic (T2- Rs. 2.70 and 4.57%), probiotic(T3- Rs. 3.56 and 6.02%) or with a combination of 0.45 (T6- Rs. 3.45 and 5.83%) and 0.18 per cent CSB (T4 - Rs. 3.39 and 5.73%), had better economic returns. While comparing probiotic with organic acid, the treatment group supplemented with probiotic *B. subtilis*and 0.09 per cent CSB (Rs. 1.71) had lower supplemental cost and leads to economic broiler production over probiotic with 0.18 per cent CSB supplementation (Rs. 3.34).

The group fed a diet containing probiotic with 0.09 per cent CSB had better economic returns which are likely to be achieved in the commercial condition where antibiotic growth promoters reported to be used in large scale at all phases (pre starter, starter and finisher) of growth in birds to overcome the pathogenic infection. With emerging antimicrobial resistance reported in human beings, the use of antibiotics in poultry and human beings is to be discontinued and replaced with alternates, based on the findings of this experiment for broiler chicken production, using probiotic *Bacillus subtilis* (100 g/ ton of diet) with 0.09 per cent of CSB.

In agreement with our results, Deepa et al. (2018) observed that supplementation of uncoated sodium butyrate (UCSB) had better economic returns when compared to the respective levels of CSB and an increase in the revenue to the tune of Rs. 1.30, 1.45 and 2.66 per kg of live weight gain in 0.18, 0.03 and 0.06 per cent UCSB supplementation in broiler diet, respectively, when compared to control and the antibiotic fed groups. In this experiment, only CSB was examined, where the supplementation of probiotic and 0.09 per cent CSB in combination (T3) was found to be better economic returns due to the better body weight gain with optimal feed intake and performed almost better to antibiotic group in terms of cost benefit.

CONCLUSION

The increase in the revenue of Rs. 4.31 and Rs. 1.61 per kg live weight gain in the group supplemented with combination of coated sodium butyrate at 0.09 per cent and *Bacillus subtilis* 100 g/ton of diet (T5) compared to basal diet and antibiotic group (T2), respectively. It could be an important observation as industry's attempts to avoid the use of antibiotics in broiler feed.

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