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Effect of calcium propionate and coated sodium butyrate as an alternative to antibiotic growth promoters on the serum profile of commercial broiler chicken

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Abstract
An experiment was conducted with 240 day old commercial broiler chicks to evaluate the effectiveness of replacing antibiotic growth promoters with organic acid salts on the serum profile. Treatment diets were supplemented with lincomycin HCl (4.4 ppm), bacitracin methylene disalicylate (BMD; 50 ppm), each two levels (ppm) of calcium propionate (1000, 2000) and coated sodium butyrate (500, 1000) either alone or in combination (1000+500) with control. The results showed that there was no significant difference in kidney & liver function tests, serum protein and mineral profile among the treatment groups. And in case of lipid profile OAs supplementation showed significantly beneficial impact. The levels of serum profile parameters were in normal range, that suggesting good nutritional status of the birds in all the treatment groups. Salts of organic acids (OAs) could be safely supplemented as an alternative to antibiotic growth promoters (AGPs) in the commercial broiler diet to lower the risk of residues in the meat and to avoid the antibiotic resistance (if any) to the consumers without impair the serum profile and birds health.

Keywords: Organic acid, Calcium propionate, Sodium butyrate, Serum lipid profile, protein profile, mineral profile, kidney and liver function tests

1. Introduction

The use of in fed antibiotics for growth promotion had been arisen with the intensification of livestock farming. Administration of sub-therapeutic level of antibiotics and antimicrobial agents is widely practiced so as to control the infectious agents and to improve the productivity of food animals. Currently, there is a controversy surrounding the use of antibiotic growth promoters (AGPs) for animals destined for meat production, as overuse of any antibiotic over a period of time may lead to resistance of these bacterial populations to the antibiotic used. Which in-turn affect human health either directly through residues of an antibiotic in meat or indirectly through the selection of antibiotic resistance determinants that may spread to human pathogen.

In 2006, the European Union imposed a complete ban on the use of antibiotics in animal feed as growth promoters. World Health Organization (WHO) along with World Organization for Animal Health (OIE) encourages the health, agriculture, veterinary sector for reducing the injudicious use of antibiotics as growth promoters and further to decrease the spread of resistant bacteria (Cogliani *et al.* 2011) [3].

Therefore, other non-therapeutic alternatives such as probiotics, prebiotics, symbiotics, antimicrobial peptides, enzymes, essential oils, eucalyptus oil, organic acids, clay minerals, egg yolk antibodies, rare earth elements, recombinant enzymes and immuno-stimulants have been introduced as an alternative to the antibiotic growth promoters.

Among the alternatives, organic acids could be considered as the best possible choice for securing the supply of safe food. Acidification increases gastric proteolysis, protein and amino acid digestibility and utilization of minerals and thus improving the performance of the birds (Haque *et al.* 2009) [6]. Organic acids (propionic acid and butyric acid) effectively replace the antibiotics and improve the production performance like body weight gain and FCR (Vijayalakshmi *et.al.* 2015; Adil *et al.* 2011; Deepa *et al.* 2016) [10, 1, 4] and had good impact on serum biochemical profile (Kamal and Ragaa, 2014) [8].

The efficiency of the calcium propionate and coated sodium butyrate was not much studied as an alternative to commonly incorporated antibiotic growth promoters such as lincomycin hydrochloride and bacitracin methylene disalicylate (BMD) in the commercial broiler chicken feed in India. Hence, the present study was undertaken to evaluate the response of calcium propionate and coated sodium butyrate at different levels either alone or in combination as an alternative to antibiotic growth promoters such as lincomycin hydrochloride and bacitracin methylene disalicylate (BMD) to assess whether it impairs the liver & kidney functions, serum protein, lipid, mineral profile of the commercial broiler chicken.

Materials and Methods

A six week biological experiment was conducted in commercial broiler chicken at Poultry Research Station, TANUVAS, MMC, Chennai. The birds were reared in brooder cages for first two weeks; then they were shifted to grower cages till the end of the experiment. The general management practices followed and the chicks were vaccinated against Ranikhet disease [RDVF (day seven), RDV-Lasota (day 28)] and Infectious Bursal Disease (Intermediate Georgia strain; day 14).

The experiment was split into pre-starter (0-14 d), starter (15-28 d) and finisher (29-42 d) periods. For this study, 240 number of unsexed day old chicks (COBB 400) were randomly distributed into eight treatment groups with three replicates each (10 chicks per replicate). They were fed with corn-soybean meal based basal diet (unsupplemented control) or the basal diet with a antibiotic growth promoters (lincomycin; 4.4 ppm and BMD; 50 ppm) or organic acids (OAs) such as calcium propionate (1000 or 2000 ppm) and coated sodium butyrate (500 or 1000 ppm) either alone or in combination (CP 1000 + SB 500) for a period of 42 days.

Serological Analyses

The blood samples were collected on the end of the trial period (two birds/ replicate). The collected blood samples were allowed to clot and centrifuged for 10 minutes at 2000 rpm to separate the serum. The separated serum was kept in -20°C until analysed. The serum samples were analysed for lipid profile, protein fraction, minerals, liver and kidney function tests in an A15 Biosystem auto analyser by using commercially available enzymatic diagnostic kits that were purchased from Agapee chemicals, Chennai.

Results and Discussion

The influence of replacing the antibiotic growth promoters with salts of organic acids in the diet on haemato-biochemicals, namely, total protein, serum lipid profile, aspartate amino-transferease (AST), alanine amino-transferease (ALT), serum creatinine, uric acid, serum calcium and serum phosphorus had been studied in the serum samples collected at the end of the experiment.

a) Protein and Lipid Profile

The results (Mean±SE) on serum total protein, albumin, globulin and lipid profile as influenced by supplementation of AGPs and OAs in the feed as growth promoters on mean serum protein and lipid profile in commercial broiler is bestowed in Table 1.

It was observed that there was a non-significant (P>0.05) influence on serum protein profile upon replacing the antibiotic growth promoters with salts of organic acids in the diet. The mean values of the total serum protein ranged from the lowest 3.15 g/dl in the group (T7) supplemented with OAs in combination (CP+SB) to the highest 3.91 g/dl in the group (T6) supplemented with sodium butyrate (1000 ppm).

Moreover supplementation prevents the elevation of serum protein fragments and the levels of protein fractions were in normal range, that suggesting good nutritional status of the birds with no pathological liver lesions in all the treatment groups. The findings of the present study are in concordance with the results reported by Mahdavi and Torki, (2009), who also found that the supplementation of organic acid would not influence the serum protein level.

b) Lipid Profile

The data pertaining to the serum lipid profile of commercial broiler chicken that were supplemented with AGPs and OAs significantly influence serum protein profile. It was noted that significant reduction (P<0.01) in the serum triglyceride level in the groups supplemented with coated sodium butyrate at 500 ppm (T5) and 1000 ppm (T6), calcium propionate at 2000 ppm (T4) than the groups such as negative control and BMD (T2). Other groups had comparable serum triglycerides among treatments. The results of the study are in harmony with the findings of Jang *et al*. (2011) [7]; Mansoub *et al*. (2011) [11]; Dehghani-Tafti and Jahanian (2016) [5]. And the groups supplemented with calcium propionate at 2000 ppm (T4; 205 mg/dl) and coated sodium butyrate 1000 ppm (T6; 207 mg/dl) had a significantly (P<0.05) reduced serum total cholesterol level than negative control (248 mg/dl) and lincomycin (T1) groups. Other groups had comparable serum cholesterol level among treatment groups. Moreover the groups supplemented with higher levels of calcium propionate (T4; 43.80 mg/dl) and coated sodium butyrate (52.96 mg/dl) had a significantly (P<0.05) reduced level of serum LDL cholesterol than control group (66 mg/dl) and other groups had a comparable serum cholesterol content. In addition to that the groups fed with higher levels of calcium propionate (T4), lincomycin (T1) significantly (P<0.05) increased the serum HDL (158.33 mg/dl) than control group (120.03 mg/dl). Other dietary groups had a comparable level of serum HDL. These results are in admittance with Clinkenbeard *et al*. (1975) [2]; Rodwell *et al*. (1976) [13] and Sarono (2003) [14].

The synthesis of cholesterol from acetyl-CoA is mediated by the cytoplasmic form of 3-hydroxy-3-methylglutary-CoA (HMG-CoA) synthase (Clinkenbeard *et al*. 1975) [2]. Middleton (1967) reiterated that propionyl-CoA is a competitive inhibitor with respect to acetyl-CoA for the active site on the HMG CoA synthase enzyme molecule. The activity of HMG-CoA reductase, the rate-limiting enzyme for cholesterol synthesis in animal tissues (Rodwell *et al*. 1976) [13], resulted in reduction in total cholesterol.

Organic acid treatment increases the *Lactobacillus* species count in caecum, which has a high bile salt hydrolytic activity, is responsible for deconjugation of bile salts (Sarono, 2003) [14]. Deconjugated bile acid are less soluble at low pH and absorbed at a reduced rate in the intestine and is more likely to be excreted in feces (Klaver and Van dermeer, 1993) [9].

c) Liver, Kidney Function Test and Serum Mineral (Calcium and Phosphorus) Profile

Mean (± SE) values of the effect of supplementation of AGPs and OAs on mean serum liver, kidney and serum mineral profile test in commercial broiler is bestowed in Table 2.

The replacement of antibiotic growth promoters with salts of organic acids in the diet did not have any impact on the parameters like ALT, creatinine, uric acid, calcium and phosphorus. More over serum calcium and phosphorus level numerically higher in sodium butyrate supplemented group (T5). Whereas ALT apparently (P>0.05) increased in the groups supplemented with AGPs and the groups supplemented with OAs irrespective of their doses other than T3 that was supplemented with calcium propionate at the dose rate of 1000 ppm.
It was observed that AST significantly (P<0.05) reduced in the group supplemented with BMD (50 ppm) and the group supplemented with calcium propionate (2000 ppm; T4). There was a comparable influence noticed among the other treatment groups.
All treatment groups showed numerically bonhomie reduction in serum creatinine level other than control groups.
The feeding of diets supplemented with AGPs and OAs irrespective of their doses did not have any impact on uric acid level in the serum, serum calcium and serum phosphorus. The average serum P content of various dietary treatments ranged from 5.87 to 7.49 mg/dl.

Table 1: Effect of in fed calcium propionate (CP) and coated sodium butyrate (SB) as growth promoters on serum protein and lipid profile of commercial broiler chicken (Mean ± SE)

Treatment (ppm)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
C; Control	3.53 ± 0.28	1.80 ± 0.14	1.73 ± 0.24	86.00^{a}± 9.64	248.96^{a}± 5.90	66.00^{a} ± 1.32	120.03^{c}± 6.41
T1; Lincomycin HCl - 4.4	3.90 ± 0.23	1.83 ± 0.06	2.06 ± 0.22	76.66^{ab}± 18.67	241.46^{ab}± 13.68	48.73^{bc}± 0.23	151.66^{ab}± 11.86
T2; BMD – 50	3.56 ± 0.21	1.91 ± 0.17	1.65 ± 0.95	78.66^{a}± 1.20	236.83^{abc}± 16.20	56.30^{abc}± 4.72	126.53^{bc}± 12.96
T3; CP – 1000	3.83 ± 0.24	1.78 ± 0.09	2.05 ± 0.20	72.33^{abc}± 8.64	214.33^{bc}± 8.78	58.06^{ab}± 5.36	136.56^{abc}± 3.56
T4; CP – 2000	3.18 ± 0.19	1.78 ± 0.05	1.40 ± 0.16	52.00^{bcd}± 3.05	205.00^{c}± 5.48	43.80^{c}± 1.85	158.33^{a}± 0.881
T5; SB – 500	3.43 ± 0.15	2.00 ± 0.11	1.43 ± 0.18	44.66^{d}± 0.81	226^{abc}± 8.62	56.66^{abc}± 6.83	143.26^{abc}± 8.45
T6; SB – 1000	3.91 ± 0.41	2.03 ± 0.12	1.88 ± 0.30	37.66^{d}± 1.45	207.00^{c}± 4.99	52.96^{bc}± 4.57	128.33^{bc}± 66.58
T7; CP - 1000 + SB – 500	3.15 ± 0.20	1.66 ± 0.08	1.48 ± 0.15	47.66^{cd}± 0.88	213^{bc}± 5.78	52.20^{bc}± 0.96	125.40^{bc}± 11.21
F value	1.404	1.161	1.667	5.109	3.033	2.949	2.658
Significance	NS	NS	NS	**	*	*	*

Means bearing different superscripts within the same column differ significantly; **Highly significant (P<0.01); * Significant (P<0.05); NS – Non significant (P> 0.05).

Table 2: Effect of in fed calcium propionate (CP) and coated sodium butyrate (SB) as growth promoters on liver and kidney function tests and serum mineral profile of commercial broiler chicken (Mean ± SE)

Treatment (ppm)	ALT (IU/L)	AST (IU/L)	Creatinine (mg/dl)	Uric acid (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
C; Control	32.33 ± 4.67	236.33^{ab}± 3.98	0.75 ± 0.39	7.68± 0.62	12.20 ± 1.20	7.26 ± 0.80
T1; Lincomycin HCl - 4.4	32.66 ± 2.24	215.00^{bc}± 15.19	0.32 ± 0.024	7.85± 1.10	11.70 ± 0.81	6.51 ± 0.50
T2; BMD – 50	35.16 ± 4.15	174.66^{c}± 17.70	0.29 ± 0.017	7.46± 0.69	11.26 ± 0.67	6.24 ± 0.18
T3; CP – 1000	31.66 ± 3.46	199.66^{bc}± 23.06	0.31 ± 0.023	7.85± 0.88	11.32 ± 0.49	6.27 ± 0.51
T4; CP – 2000	36.66 ± 4.33	170.33^{c}± 2.84	0.26 ± 0.011	7.25 ± 0.62	12.20 ± 0.48	6.32 ± 0.16
T5; SB – 500	44.00 ± 8.89	274.66^{a}± 18.66	0.30 ± 0.032	6.72± 1.29	12.98 ± 0.61	7.49 ± 0.17
T6; SB – 1000	37.66 ± 8.28	253.33^{ab}± 1.76	0.30 ± 0.052	9.82± 0.62	11.98 ± 0.32	6.34 ± 0.17
T7; CP - 1000 + SB – 500	32.66 ± 1.94	202.00^{bc}± 32.21	0.26 ± 0.032	7.49± 0.96	11.78 ± 0.46	5.87 ± 0.19
F value	0.601	4.043	1.315	2.170	0.656	1.836
Significance	NS	**	NS	NS	NS	NS

Means bearing different superscripts within the same column differ significantly; **Highly significant (P<0.01); * Significant (P<0.05); NS – Non significant (P> 0.05).

Conclusion
Based on the data obtained from serum analysis, salts of organic acids (OAs) such as calcium propionate and coated sodium butyrate could be safely supplemented as an alternative to antibiotic growth promoters (AGPs) in the commercial broiler diet without impair kidney & liver functions serum lipid, protein, mineral profile. Moreover supplementation prevents the elevation of serum protein fragments and the levels of protein, lipid, mineral, fractions and favourably total cholesterol and LDL significantly reduced due to the dietary treatment of calcium propionate (T4) and coated sodium butyrate (T6) than control and there was a significant (P< 0.05) increase in HDL concentration in groups fed with calcium propionate (T4) than control, T2 (BMD), T6 (SB 1000) and T7 (CP+SB). AST significantly decreased in the groups fed with calcium propionate at 2000 ppm (T4) when compared to control in addition to that they were in normal range, that suggesting good nutritional status of the birds with no pathological liver and kidney lesions in all the treatment groups.
Salts of organic acids (OAs) could be safely supplemented as an alternative to antibiotic growth promoters (AGPs) in the commercial broiler diet to lower the risk of residues in the meat and to avoid the antibiotic resistance (if any) to the consumers.

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