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Effect of Dietary Supplementation of Lauric Acid on Blood Biochemical Constituents, Nutrient Retention and Gut Microflora Count in Commercial Broilers

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ABSTRACT

Background: Ban on utilization of antibiotic growth promoters (AGP) in poultry diets has driven the implementation of alternative strategies to maintain gut health, optimal digestibility and performance in broiler production. Lauric acid (LA) is a medium chain fatty acids and could be used as a potential alternative to AGP in broiler diets. Hence, an experiment was conducted to evaluate the effect of dietary supplementation of LA at graded levels on nutrient retention, blood biochemical constituents and gut microflora count.

Methods: Three hundred, day old commercial broiler chicks were randomly allotted to 6 dietary treatments with 10 replicates of 5 chicks in each. Corn-soybean meal based basal diet (BD) was formulated for pre-starter (1-2weeks), starter (3-4 weeks) and finisher (5-6 weeks) phases without AGP (negative control). Positive control diet was the BD having 0.035% chlortetracycline as AGP. The remaining 4 experimental diets were formulated by supplementing LA to BD at rate of 0.05% (LA-50), 0.075% (LA-75), 0.1% (LA-100) and 0.2% (LA-200), respectively. Blood was collected from 1 bird per replicate on 35thd to estimate blood biochemical constituents. To assess the nutrient retention, metabolic trial of 3 d duration was conducted on one randomly selected bird per replicate after 42 d. On same day, 1 bird from each replicate was sacrificed for enumeration of total bacteria and *Escherichia coli* in gut.

Result: The utilization of dry matter was higher (P<0.05) in broilers fed on graded levels of LA than BD fed chicks and was comparable to that of AGP fed birds. Broilers supplemented with LA-200 and LA-75 diet had higher (P<0.01) crude protein retention than those fed BD. Significantly (P<0.05) higher ether extract retention was observed in LA-200 fed birds compared to those on BD, AGP, LA-50, LA-75 and LA-100. Replacement of AGP with LA (0.05 to 0.2%) in diets decreased (P<0.05) total bacterial and *E.coli* count in gut. Serum glucose, cholesterol, albumin, globulin and its ratio were not affected while serum total protein was higher (P<0.05) in LA-75 and LA-200 fed birds compared to BD birds. Based on the results it could be concluded that, lauric acid supplementation at 0.05% in diets improved nutrient retention and gut health and could be used as alternative to antibiotic growth promoter.

Key words: Blood biochemical constituents, Broilers, Lauric acid, Nutrient retention.

INTRODUCTION

Gut health plays an important role in improving the bird health and productivity as it is the major site for feed digestion, nutrient absorption and metabolism, energy and protein utilization (Rinttila and Apajalahti, 2013). The microbial balance in gut is of utmost importance in maintaining gut health (Gabriel *et al*., 2006). The antibiotics growth promoters (AGPs) at sub optimal dosage were widely used as feed additive to have good microbial balance in gut and improve performance of the birds. But use of these AGPs has been banned worldwide because of public health concern over antibiotic residual effects and the development of drug resistance bacteria. Therefore has compelled the researchers to explore the alternatives for AGPs which are environmental friendly and safe for both animal and humans (Cabuk *et al.*, 2006). Many such prominent alternatives were prebiotic, probiotic, synbiotic and organic acids that beneficially affect the host by improving its intestinal microbial balance (Kolida and Gibson, 2011). Organic acids include short chain fatty acids (SCFA) and medium chain fatty acids (MCFA) that produce the antibacterial effects and modify the gut conditions by reduction of its pH, promoting proteolytic enzyme activities, creating stability of the microbial population, stimulating the growth of beneficial bacteria and enhance nutrient digestibility (Dittoe *et al*., 2018). Due to their multi roles, organic acids are being widely accepted as alternative to AGPs and European Union (EU) approved organic acids

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as safe and can be used as the feed additives in poultry production (Canibe *et al.,* 2001). Furthermore, it has been found that MCFAs are more bactericidal to numerous gram-negative and gram-positive bacteria than the SCFAs (Hermans *et al.*, 2010).

Most of the research conducted was to evaluate the effect of short-chain fatty acids as alternative to AGP and only few studies were conducted on evaluating medium chain fatty acids (C_6-C_{12}) as organic acids in poultry diets. Therefore, present study was undertaken to evaluate the effect of dietary supplementation of lauric acid (C12 saturated fatty acid) as an alternative to AGPs on nutrient retention, blood biochemical constituents and gut microflora count in commercial broilers.

MATERIALS AND METHODS

Site of study

The experiment was carried out at the College of Veterinary Science, PVNR Telangana Veterinary University, Rajendranagar, Hyderabad, India. The experimental protocols were done with the approval of Institute's Animal Ethics Committee (IAEC) of PVNR Telangana Veterinary University, Hyderabad, 500 030, Telangana, India.

Birds and management

Three hundred, day old commercial broiler chicks (Cobb-400) were randomly allotted to 6 dietary treatments with 10 replicates of 5 chicks in each separately housed in randomly positioned identical-sized four-floor electrically heated battery cages having raised wire floors and fitted with feeder, water and a droppings tray underneath. The chicks of all groups were kept under uniform management and standard hygienic conditions throughout the experimental period. Birds were immunized for New Castle disease on 7th and 28th day of age with Lasota vaccine and for infectious bursal disease on 14thand 21st day of age with Georgia strain vaccine. All replicate groups of chicks were offered the respective diets *ad libitum*. Clean and fresh drinking water was provided *ad libitum* daily.

Diets

A corn-soybean meal based basal diet (BD) was prepared for pre-starter (1 to 2weeks), starter (3 to 4 weeks) and finisher (5 to 6 weeks) phases without antibiotic growth promoter (Negative control) (Table 1). Positive control diet was the BD having 0.035% chlortetracycline as AGP. Subsequently four experimental diets were formulated by supplementing lauric acid to BD at rate of 0.05% (LA-50), 0.075% (LA-75), 0.1% (LA-100) and 0.2% (LA-200), respectively.

Gut bacterial count and blood biochemical constituents

On 35th d of feeding, one bird from each replicate was sacrificed by cervical dislocation and intact gastrointestinal tract from the esophagus to the caecum was separated and placed in sterile petri dishes after washing with normal saline to remove exterior blood and tissue debris. The digesta was taken from 5 cm upper side of 'Meckel's diverticulum' in pre-weighed sterile Eppendorf tube. To get decimal dilution, 1 gram of digesta was taken in sterile test tube and diluted with 9 ml of normal physiological saline. Using separate sterile pipets serial dilution of each sample were made and 0.1 ml diluted digesta was pipette out into 2 separate marked sterile disposable petri dishes (Hi Media Laboratories Ltd., Mumbai, India) and to this 12-15 ml plate count agar (Hi Media Laboratories Ltd.) was poured for enumeration of total bacteria. While 12-15 ml Eosin Methylene Blue agar (Hi Media Laboratories Ltd.) was poured in another 2 separate petri plates to enumerate *Escherichia coli* (AOAC, 2012). After solidification of agar, inverted solidified Petri dishes were incubated for 48±2 h at 35°C. The colonies were enumerated using a colony counter (manufactured by Optics Technology, New Delhi, India) and the results were expressed as $\log_{10}$Colony Forming Units (CFU)/g digesta. Aseptically, 3 ml of blood was collected from one bird per replicate on 35th day and collected serum samples for determining concentration of serum biochemical constituents *viz*., glucose, cholesterol, total protein, albumin and globulin by using diagnostic kits.

Metabolic trial

A metabolic trial of 3 days duration was conducted on 42nd d of feeding on selecting one bird randomly from each replicate to arrive at dry matter (DM), crude protein (CP) and ether extract (EE) utilization to ensure the emptying of gastrointestinal tract. The birds were fasted for 17 hours (4.00 PM to 9.00 AM) prior to collection period. The daily feed intake and faeces voided were recorded. Representative samples of feed offered, residue left and faeces voided were analyzed for DM, EE and CP concentrations (AOAC, 2012).

Statistical analysis

The results obtained were subjected to analysis through software (version 16.0; SPSS, 2007) by applying one way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test (Duncan, 1955) with a test of significance at 5%. All the statistical procedures were done as per the procedures of Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Nutrient retention

The utilization of dry matter (DM) was higher ($P<0.05$) in broilers fed diets supplemented with the graded levels of lauric acid (0.05-0.2%) than BD and was comparable with those fed AGP containing diet. Broilers supplemented with LA-200 and LA-75 diet had higher ($P<0.01$) crude protein (CP) retention than those fed BD. Broilers fed diets supplemented with LA-50, LA-100 and AGP had intermediate CP retention compared to LA-200 and BD. Higher ether extract retention was observed in LA-200 fed birds compared to those BD, AGP, LA-50, LA-75 and LA-100. The ether extract retention in LA-50, LA-75 and LA-100 was comparable with that of AGP fed birds (Table 2).

Increase in nutrient retention with LA supplementation might be due to fact that MCFAs reduces the gastric pH which activates pepsinogen and other zymogens by adjusting gastric acidity closer to that required for optimal digestion activity (Jongbloed *et al.,* 2000). Beside this, MCFAs, stimulates cholecystokinin secretion in the small intestine which in turn, stimulates the secretion of pancreatic protease (Stubbs and Stabile, 1985) and this increased enzyme activity could have improved the digestion of proteins and possibly other nutrients observed in present study. Apart from these, MCFAs has a strong antimicrobial effect on the intestinal microarchitecture (Dierick *et al.,* 2002) which decreases competition between host and microbes thereby ultimately increasing the availability of nutrients for absorption and utilization as observed in current study (Table 4). Similarly, Chotikatum *et al.* (2009) reported significantly ($P<0.05$) higher digestibility of protein in broilers with supplementation of medium chain fatty acids (C6, C8 and C10) at 1: 1,000 in water than those given normal tap water. Similarly, improved DM, CP, EE, CF and NFE have been reported by many researchers with dietary supplementation of other organic acids such as mixture of propionic acid, fumaric acid, citric acid and sorbic acid (Thirumeignanam *et al.,* 2006), formic acid (Adeniji *et al.,* 2015), mixture of acetic acid, butyric acid, citric acid and formic acid (Ndelekwute *et al.,* 2016) and organic acid blend (formic and propionic acids) (Basmacioğlu-Malayoğlu *et al.,* 2016).

Serum biochemical constituents

Dietary supplementation of lauric acid ranging from 0.05 to 0.2% or AGP had no effect on serum glucose and cholesterol (Table 3). In line with present findings, many experiments on dietary organic acid supplementation have indicated no effects on serum glucose and cholesterol levels. No effect on serum glucose and cholesterol was observed in broilers fed basal diet or BD supplemented with formic acid @ 0.05 or 0.1% or avilamycin @ 11ppm (Hernández *et al.,* 2006) or in layers fed BD supplemented

Table 1: Ingredient and nutrient composition of standard diets fed to broilers.

Ingredient (g/kg)	Pre-Starter(1-14 days)	Starter(15-28 days)	Finisher(29-42 days)
Maize	539.86	561.51	606.05
Soyabean meal	382.65	345.71	294.66
Rice bran oil	34.86	49.46	59.23
Salt	4.24	4.24	4.23
Dicalcium phosphate	17.47	17.97	15.65
Lime stone powder	12.19	12.18	11.73
DL-Methionine	2.77	2.74	2.34
L-Lysine HCl	1.73	1.61	1.29
L-Threonine	0.12	0.46	0.67
L-Tryptophan	0	0.02	0.06
Vitamin AB_2D_3K[1]	0.15	0.15	0.15
Vitamin B complex[2]	0.15	0.15	0.15
Choline chloride-75%	0.6	0.60	0.60
Toxin binder	1.0	1.00	1.00
Trace mineral mixture[3]	1.2	1.20	1.20
Coccidiostat	0.50	0.50	0.50
Total	1000.0	1000.0	1000.0
Nutrient composition (calculated)			
Metabolisable energy (kcal/ kg)	3000	3100	3200
Crude protein (%)	22.5	21.0	19.0
Calcium (%)	0.90	0.90	0.82
Non-phytate phosphorus (%)	0.45	0.45	0.40
L- Lysine (%)	1.25	1.15	1.00
DL- Methionine (%)	0.57	0.55	0.49
L- Threonine (%)	0.77	0.75	0.70
L-tryptophan (%)	0.225	0.210	0.19
Sodium (%)	0.18	0.18	0.18

[1]Each gram of Vitamin AB_2D_3K contained: Vitamin A- 1,00,000 IU, Vitamin B_2-50 mg,Vitamin D_3- 20,000 IU, Vitamin K-20 mg, Anti-oxidant-200ppm

[2]Each gram of Vitamin B complex contained: Vitamin B_1 5 mg, Vitamin B_6 8 mg, Vitamin B_{12} 50 mg, Niacinamide 6 mg, Calcium Pantothenate 45 mg and Folic Acid 45 mg

[3]Trace mineral mixture provided per kg diet: Manganese 110 mg, Zinc 100 mg, Iron 110 mg, Copper 20 mg, Iodine 2.5 mg, Cobalt 1mg, Chromium 0.4 mg and Selenium 0.15 mg.

with organic acid mixture (60% formic acid, 20% propionic acid and 20% soft acid) (Kaya *et al*., 2015). Similarly, supplementation of mixture of butyric, fumaric and lactic acid either at 2 or 3% of diet showed no significant (P>0.05) difference in the concentration of serum glucose and cholesterol compared to control group (Adil *et al.*, 2010). In another study, serum glucose and serum cholesterol levels remain unaffected with dietary supplementation of formic acid and propionic acid or their combination at 0.1 or 0.15% (Venkatasubramani *et al.,* 2014), formic acid at 0.05% (Pathak *et al.*, 2016), acetic acid at 1, 2, 4, 8 and 10% (Seifi *et al*., 2015) and benzoic acid at 0.4 or 0.8% (Hassan and Raheem, 2016) in broilers. Contrary to the present findings, supplementation of 0.1, 0.2 and 0.3% MCFA (balance mixture of C8-10) lowered serum concentration of glucose, LDL-cholesterol and total cholesterol (Shokrollahi *et al.*, 2014). Balanced mixture of (C8-10) MCFAs supplemented either at 0.1 or 0.2% butyrate or combination of butyrate and MCFAs significantly (P<0.01) decreased serum glucose and cholesterol level (Khatibjoo *et al.*, 2017).

The serum total protein in birds fed LA diets at either of the concentration was comparable to AGP diet fed birds, while the concentration was higher (P<0.05) in LA-75 and LA-200 birds compared to BD birds. No effect was observed on serum albumin and globulin levels with lauric acid supplementation (Table 3) corroborating with the findings of Abdalla *et al.* (2013). Similarly, serum albumin, protein and globulin levels were not affected with dietary supplementation of either balanced mixture of MCFA (60% C6 to C12) @ 0.3 and 0.6% or Neomycin (0.2 and 0.4%) (Fasanmi *et al.*, 2015) in broilers or supplementation of organic acid mixture (60% formic acid, 20% propionic acid and 20% soft acid) @ 1.5, 3.0 and 4.5g/kg in layers (Kaya *et al*., 2015).

Total bacterial and *Escherichia coli* count

Total bacterial and *Escherichia coli* count (log $_{10}$ CFU/g) differed significantly (P<0.05) among the groups (Table 4). All lauric acid supplemented groups showed significantly (P<0.05) lower total bacterial count compared to BD and AGP fed groups. Similar trend of total bacterial count was observed on *E. coli* count in gut. Dietary supplementation of lauric acid resulted in lowered (P<0.05) *E. coli* count in the gut of broiler chicks compared to either AGP or BD fed birds, with no difference among the LA group birds, attributed to antimicrobial activity of MCFA. Furthermore, transmission electron microscopy (TEM) studies conducted by Shilling

Table 2: Effect of dietary supplementation of lauric acid on apparent nutrient retention (%) in broilers.

Diet	Dry matter	Crude protein	Ether extract
Basal diet (BD)	72.37^{b}	68.37^{c}	76.95^{c}
AGP	74.81^{a}	69.63^{bc}	78.04^{bc}
LA-50	75.20^{a}	70.65^{bc}	79.47^{ab}
LA-75	75.65^{a}	72.74^{ab}	78.63^{bc}
LA-100	75.51^{a}	69.98^{bc}	78.52^{bc}
LA-200	76.93^{a}	75.12^{a}	80.99^{a}
N	10	10	10
SEM	0.368	0.523	0.343
P-value	0.01	0.001	0.015

^{abc}Means with different superscript in a column differ significantly: P<0.05, P<0.01, P- value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean; AGP: BD with 0.035% antibiotic growth promoter; LA-50: BD with 0.05% lauric acid; LA-75: BD with 0.075% lauric acid; LA-100: BD with 0.1% lauric acid; LA-200: BD with 0.2% lauric acid.

Table 3: Effect of dietary supplementation of lauric acid on serum biochemical constituents in broilers.

Diet	Glucose (mg/dl)	Cholesterol (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin Globulin ratio (A:G)
Basal diet (BD)	173.2	160.8	4.93^{b}	1.57	3.36	0.47
AGP	167.9	159.8	5.10^{ab}	1.87	3.23	0.60
LA-50	177.6	156.9	5.16^{ab}	1.74	3.42	0.52
LA-75	175.4	162.4	5.30^{a}	1.77	3.53	0.51
LA-100	172.2	164.3	5.10^{ab}	1.93	3.17	0.62
LA-200	171.0	171.8	5.21^{a}	1.97	3.24	0.72
N	10	10	10	10	10	10
SEM	1.445	1.843	0.035	0.047	0.057	0.037
P-value	0.494	0.268	0.050	0.132	0.453	0.391

^{ab}Means with different superscript in a column differ significantly: P<0.05, P<0.01, P- value: probability value.Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean; AGP: BD with 0.035% antibiotic growth promoter; LA-50: BD with 0.05% lauric acid; LA-75- BD with 0.075% lauric acid; LA-100: BD with 0.1% lauric acid; LA-200: BD with 0.2% lauric acid.

Table 4: Effect of dietary supplementation of lauric acid on microbial counts (log $_{10}$ CFU/g) in gut of broilers.

Diet	Total bacterial count (log $_{10}$ CFU/g)	*Escherichia coli* count (log $_{10}$ CFU/g)
Basal diet (BD)	7.110[a]	6.784[a]
AGP	7.078[a]	6.688[b]
LA-50	6.981[b]	6.602[c]
LA-75	6.956[b]	6.605[c]
LA-100	6.974[b]	6.616[c]
LA-200	6.957[b]	6.598[c]
N	10	10
SEM	0.009	0.011
P-value	0.001	0.001

[abc]Means with different superscript in a column differ significantly: P<0.05, P<0.01, P- value: probability value. Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean; AGP: BD with 0.035% antibiotic growth promoter; LA-50: BD with 0.05% lauric acid; LA-75: BD with 0.075% lauric acid; LA-100: BD with 0.1% lauric acid; LA-200: BD with 0.2% lauric acid.

(2013) revealed that lauric acid damaged the cytoplasmic structure of bacteria which leads to death of bacteria. Present study results are in accordance with Skrivanova *et al.* (2015) who observed that supplementation of 0.25 or 0.5 % caprylic acid in diets of broilers resulted in reduced (P<0.05) salmonellas count of crop and cloacal contents compared to control group. Jadhav *et al.* (2021) observed reduction in total bacterial count with numerical reduction in coliform count with dietary supplementation of 0.25% lauric acid in broilers. Other studies also reported that intestinal bacterial population was reduced in broilers with dietary supplementation of 0.5 or 1.0% of sodium butyrate or calcium propionate (Vinus *et al.,* 2018), phenyl lactic acid (Kim *et al., 2014),* citric acid, lactic acid and phosphoric acid (Sultan *et al.,* 2015), 0.3% C10 and 2.7% C12 acids (Van der Hoeven-Hangoor *et al.,* 2013) and blends of lactic acid and citric acid (Natsir *et al.,* 2017).

CONCLUSION

Lauric acid supplementation helps in improving nutrient retention, gut health and serum protein without affecting other blood biochemical constituents and can be replacer for AGPs. Based on the results it could be concluded that, lauric acid supplementation at 0.05% in diets improved nutrient retention and gut health with no further beneficial effect at higher doses (0.075-0.2%) and could be used as alternative to antibiotic growth promoter in diets of broilers.

Conflict of interest

The authors declare that they have no conflict of interest.

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