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Dietary Inclusion of Feed Additives in Broilers: Effect on Carcass Characteristics, Visceral and Lymphoid Organs and Gut Health

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ABSTRACT

The present experiment was conducted on 288-day-old unsexed broiler chicks to evaluate the effect of dietary feed additives viz. neem leaf meal (NLM) and citric acid (CA) on carcass characteristics, visceral and lymphoid organs, and gut health in broiler chickens. The chicks were randomly distributed into 6 dietary groups of 48 birds per group, each having 4 replicates (12 birds per replicate) in a randomized block design. The birds were fed on a standard diet without any supplementation control (T0); and with supplementation of NLM 2.5 g/kg of feed (T1); CA 15 g/kg of feed (T2); CA 25 g/kg of feed (T3); NLM 2.5 g + CA 15 g/kg of feed (T4); NLM 2.5 g + CA 25 g/kg of feed (T5). Eight birds from each treatment (2 per replicate) were randomly selected for slaughter and collection of samples and microbial study at day 42. The yield of the carcass, cut-up parts, and visceral organs, and length of the small intestine revealed comparable values without any adverse effect in the groups. The caecal E. coli population reduced significantly ($P<0.05$) in treatments T2 and T3 as compared to T0. The lower ($P<0.05$) caecal pH was observed in groups T1, T2, T3, T4, and T5 than T0. It can be concluded that feeding CA at 15 g and 25 g/kg of feed improved gut health without any adverse effect on the carcass and internal organs. Hence, supplementation of CA at two dietary levels i.e. 15 g and 25 g per kg of feed could be an alternative to produce healthy chickens.

Key words: Broiler, Carcass, Caecal pH, Citric acid, Gut health, Neem leaf meal

INTRODUCTION

Chickens are the major source of high-quality cheaper animal proteins in a shorter time span. At present, the demand for such cost-effective and high-valued food has been raised enormously due to the ever-growing human population, shifting food habits, and improved purchasing ability of consumers across the world. Hence, to bridge up the gap between demand and supply, cost-effective broiler production using feed additives (Huyghebaert *et al.,* 2011) is of paramount importance. The balanced feed followed by efficient nutrient utilization has significantly exploited the production potential of chickens. Among several factors, 'gut health' is the most vital which significantly affects the productivity and feed efficiency of broilers (Gracia *et al.,* 2007). In poultry, gut microbiota regulates intestinal health by modulating host physiology, mainly through the competitive exclusion of harmful microbes by preventing colonization (Diaz Carrasco *et al.,* 2019). The poultry gut is a vital organ system and plays a key role in digestion and assimilation of feed nutrients besides, it also regulates the immune system of the birds. Apart from nutrient absorption, intestinal mucosa acts as an effective barrier between lumen contents and host splanchnic tissues, and hence; it is a key determinant of gut health and performance of birds (Rinttila and Apajalahti, 2013). For proper functioning of the mucosal barrier, a dynamic balance between mucus layer, epithelial cells, microbiota, and immune cells is essential which might be influenced by diet and microbes affecting the health and productivity of chickens (Sugiharto, 2016).

Currently, a higher growth rate and efficient feed

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conversion are two major goals of the broiler industry. To balance the gut ecosystem for higher growth (Huyghebaert *et al.,* 2011) a subclinical level of antimicrobials has been widely practiced in the poultry sector (Murugesan *et al.,* 2015). But currently, indiscriminate use of antibiotic growth promoters in the poultry industry has been widely criticized due to the development of anti-microbial resistance and transfer of antibiotic resistance genes from animal to human microbes, posing potential threats to human health. Further, withdrawal of such antimicrobial feed additives from poultry feeding reduced the production and increased morbidity (Huyghebaert *et al.,* 2011) and mortality in commercial flocks (Dibner and Richards, 2005). Hence, after the ban on the use of antimicrobials in food species by the European Union, researchers are compelled to explore alternative growth promoters (Pearlin *et al.,* 2020). At present, the broiler industry is looking for some non-therapeutic feed additives to replace the antibiotics for achieving higher gains through efficient feed conversion and less input cost. Therefore, keeping in view the above-cited problems and significance of novel feed additives in poultry, the present study was designed to evaluate the impact of dietary NLM and CA on the carcass, visceral and lymphoid organs, and gut health in broilers.

MATERIALS AND METHODS

The present experiment was conducted at Instructional Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari Agricultural University, Campus, Navsari, Gujarat. In this study, two different feed additives like NLM and CA were utilized to formulate experimental diets as per standards of BIS (2007) to meet their energy and protein requirements during the pre-starter, starter, and finisher phases (Table 1). To prepare NLM, medium-sized fresh green neem leaves were harvested within the university campus, dried in a hot air oven (600C for 12 h), and milled to a fine powder. The CA, a crystalline powder was supplied by Aashi Chem, Surat, India. The experimental diets were prepared by incorporating and proper mixing of NLM and CA as per the experimental protocol. The proximate analysis of experiential diets was carried out to determine the dry matter, crude protein, crude fibre, ether extract, total ash, and nitrogen-free extract (Table 1) as per methods described in AOAC (2011).

Table 1. Ingredients and chemical composition of experimental broiler diets

	Experimental diet		
Ingredient (%)	**Pre-starter**	**Starter**	**Finisher**
Maize	51.300	53.900	54.080
De-oiled rice bran	1.000	1.150	9.170
De-oiled soybean cake	42.300	39.550	30.600
Calcite powder	2.235	2.235	1.650
Di-calcium phosphate	2.000	2.000	1.350
Trace mineral*	0.100	0.100	0.000
Lysine	0.100	0.100	0.100
D.L.-methionine	0.135	0.135	0.100
Metabolic activator	0.100	0.100	0.100
Enzyme	0.050	0.050	0.050
Salt	0.300	0.300	0.400
Toxic binder	0.100	0.100	0.100
Maduramycine-1%	0.050	0.050	0.050
Furazolidone	0.030	0.030	0.050
Vegetable oil	0.000	0.000	2.000
Vitamin and mineral supplement**	0.200	0.200	0.200
Chemical composition (%)			
Crude protein	26.89	22.89	21.61
Crude fiber	4.21	4.18	4.32
Total ash	6.53	5.83	6.69
Nitrogen free extract	58.1	61.9	61.0
Ether extract	4.28	5.21	6.35

*Trace minerals: Each kg contains: Copper-15 g, Iodine-1 g, Iron-60 g, Manganese- 80 g, Selenium- 0.3 g, Zinc- 80 g; Metabolic activator: Lecithin extract treated with co enzyme; Enzymes: Each gram contains: Xylanase-2000 IU, Amylase-400 IU, Protease- 4000 IU, Cellulase-500 IU; Toxin Binder: Selected silicates, surfactants, organic acids and salts of organic acids; **Vitamin and mineral supplement: Each 2 kg contains: Vit. A-50 lakh IU, Vit. B2- 2 g, Vit. B6- 0.4 g, Vit. B12- 5600 mcg, Vit. E- 800 IU, Iron- 7.5 g, Vit. D3- 6.25 lakh IU, Choline chloride- 10 g, Copper- 2 g, Iodine- 1 g, Zinc- 15 g, Manganese- 27.5 g, Calcium- 27.25 %, phosphorus- 7.45 %, Calcium pantothenate- 4 g.

A total of 288 day-old vaccinated straight run broiler chicks (Vencobb- 400) were procured from Venky's Hatchery Pvt. Ltd., Anand. These were weighed individually and randomly allocated into six dietary groups based on their body weight. Each group comprised of 48 chicks was further replicated 4 times with 12 chicks per replicate in randomized block design. The grouped birds were fed on a standard diet without any supplementation control (T0); and with supplementation of NLM 2.5 g/kg of feed (T1); CA 15 g/kg of feed (T2); CA 25 g/kg of feed (T3); NLM 2.5g + CA 15 g/kg of feed (T4) and NLM 2.5 g + CA 25 g/kg of feed (T5).

The experimental birds were raised on deep litter (rice husk) with standard managemental practices for floor space, temperature, relative humidity, ventilation, lighting, feeding, watering, and other routine bio-security aspects. Brooding temperature maintained at 34±1°C up to 7 days of age and later on decreased by 3°C per week till it reaches 21°C. A photoperiod of 24 h was provided to the entire flock throughout the experiment. The experimental birds were fed ad-lib using experimental diets. A measured quantity of feed and water was offered twice daily at 08:00 and 18:00 h throughout the experimental period. At the end of the experiment (42 days of age), 2 birds per replicate were randomly selected, fasted overnight, and sacrificed through cutting carotid arteries and partial slicing of the neck. The following measurements were taken to examine the carcass and visceral organs such as pre-slaughter live weight, eviscerated weight, shank, head, neck, liver, heart, empty gizzard, pancreas, spleen, thymus and bursa abdominal fat pad and empty intestine weights, etc. All these were calculated as such (in grams) and as a percent of the live weight of the bird. The different segments of the small intestine like duodenum, jejunum, and ileum were dissected and washed in normal saline to record their length and mass. To measure the pH and microbial counts, samples of caecal contents (1-2 g) were collected into a 2 ml safe lock Eppendorf tube and kept on ice until they were analyzed. The population of E. coli was estimated as the log10 of colony-forming units (cfu) per gram of caecal contents. The E. coli was cultured with Eosin Methylene Blue (EMB) agar (Himedia Laboratories Pvt. Ltd., Mumbai, India) at 37°C for 48 h under aerobic conditions. The colonization of E. coli on EMB agar was indicated by a distinctive metallic green sheen due to the metachromatic properties of the dyes. The colonies of E. coli were counted under the digital colony counter and expressed appropriately (log10×106 cfu/g). After sampling for enumeration of microbial population, caecal contents were drained into a small beaker for estimation of caecal pH with help of a digital pH meter (PHTestr 30, Eutech Instruments Oakton, Thermo Fisher Scientific, USA).

The data were subjected to analysis of variance using Software Package for Social Sciences (SPSS, trial version 20.0, Chicago, USA) and the corresponding means were compared by Tukey-Krammer test at P <0.05.

RESULTS AND DISCUSSION

The perusal of Table 2 revealed that broilers supplemented with NLM and CA did not influence the carcass traits, which might be attributed to the fact that carcass traits are mostly governed by the genotype of individual and not much affected by the environment. Hence, these are considered as the most consistent attributes and least affected by dietary interventions. In agreement with these results, previous studies reported that supplementation of NLM (Egbeyale *et al.,* 2020; Ubua *et al.,* 2019), aqueous fresh neem leaf extract (NLE) (Shaahu *et al.,* 2020) and organic acid (OA) (Kopecký *et al.,* 2012) did not influence live and dressed body weight in broilers. However, contradictory findings were reported by Obun *et al.* (2013) and Hassan *et al.* (2016) in broilers supplemented with NLM and CA, respectively. The nonresponsive impact of NLM and CA on carcass yield (Table 2) is supported by several authors in broilers supplemented with NLM (Shihab *et al.,* 2017; Egbeyale *et al.,* 2020), aqueous fresh NLE (Shaahu *et al.,* 2020), neem seed oil (Sonhafouo *et al.,* 2019), CA (Hassan *et al.,* 2016) and OA (Fathi *et al.,* 2016) in their diets. However, in contrast to our findings, several researchers stated that feeding of NLM (Singh *et al.,* 2015), NLE (Paul *et al.,* 2020), and OA (ELnaggar *et al.,* 2017), significantly improved the carcass yield of broilers. The yield of cut-up parts did not differ significantly among the groups (Table 2), which is in line with the previous studies which indicated that supplementation of NLM (Ubua *et al.,* 2019), aqueous fresh NLE (Shaahu *et al.,* 2020), and CA (Kopecky *et al.,* 2012) had no impact on the weight of cut-up parts in chickens. Inconsistently, Singh *et al.* (2015) observed higher cut-up parts yield in guinea fowl supplemented with neem leaf powder and increased breast weight was reported by Fik *et al.* (2020) in broilers supplemented with CA.

Table 2. Effect on carcass characteristics of broilers supplemented with NLM and CA

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
Live wt. (g/bird)	2550±64	2625±60	2650±45	2587±46	2643±40	2612±83	0.83
Dressed wt. (g/bird)	1731±43	1768±57	1842±39	1762±33	1785±43	1825±69	0.66
Carcass yield (%)	67.8±0.15	67.3±0.75	69.5±0.94	68.1±0.38	67.5±0.96	69.8±1.93	0.44
Head wt. (g)	52.8±1.78	53.7±1.23	55.5±2.37	52.0±0.84	53.3±1.38	54.5±3.09	0.80
Relative head wt. (LW %)	2.08±0.11	2.04±0.02	2.09±0.07	2.01±0.04	2.01±0.04	2.08±0.08	0.91
Leg wt. (g)	89.8±5.58	82.2±2.13	94.8±5.88	86.3±2.90	89.0±3.85	98.0±5.71	0.29
Relative legs wt. (LW %)	3.54±0.29	3.13±0.09	3.57±0.17	3.34±0.15	3.36±0.09	3.74±0.14	0.23
Cut up parts							
Neck wt. (g)	70.1±3.33	73.6±2.44	79.1±2.20	71.6±4.46	74.0±2.74	76.1±3.16	0.46
Relative neck wt. (LW %)	2.74±0.10	2.80±0.08	2.99±0.11	2.76±0.13	2.79±0.07	2.91±0.12	0.61
Back wt. (g)	179.8±9.2	177.7±7.8	189.8±6.8	180.5±11.	184.2±15.3	179.0±8.5	0.96
Relative back wt. (LW %)	7.04±0.25	6.76±0.23	7.16±0.23	7.00±0.56	6.94±0.48	6.85±0.26	0.97
Thigh wt. (g)	255.7±4.7	27.50±8.9	266.3±14.2	251.6±13.21	268.7±11.86	257.3±9.15	0.68
Relative thigh wt. (LW %)	10.03±0.15	10.49±0.16	10.04±0.44	9.74±0.60	10.15±0.32	9.85±0.19	0.78
Breast wt. (g)	511.7±17.3	539.7±16.6	564.0±17.8	506.8±6.9	486.1±31.6	492.3±11.8	0.09
Relative breast wt.	20.0±0.56	20.5±0.35	21.2±0.52	19.6±0.59	18.3±1.05	18.9±0.98	0.09
Drumstick wt. (g) (LW %)	219.2±9.14	217.1±9.49	234.8±2.82	227.3±3.13	237.0±6.94	229.5±8.52	0.33
Relative drumstick wt. (LW %)	8.59±0.27	8.26±0.25	8.87±0.26	8.78±0.06	8.95±0.15	8.80±0.39	0.53
Wing wt. (g)	59.2±2.03	65.1±2.55	69.6±4.72	65.2±2.24	62.7±1.27	64.2±1.85	0.29
Relative wing wt. (LW %)	2.32±0.08	2.48±0.07	2.62±0.17	2.52±0.12	2.37±0.04	2.46±0.10	0.48

T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T_3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

Table 3. Effect of NLM and CA supplementation on visceral organs yield in broilers

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
Liver wt. (g)	45.0±2.09	45.6±1.43	46.8±1.88	43.2±2.24	46.7±2.42	40.8±4.27	0.496
Relative liver wt. (LW %)	1.76±0.05	1.74±0.08	1.76±0.06	1.66±0.06	1.77±0.10	1.55±0.14	0.409
Heart weight (g)	13.5±0.79	14.1±0.47	14.1±0.72	13.7±0.52	13.0±0.54	13.1±0.69	0.658
Relative heart wt. (LW %)	0.52±0.03	0.53±0.02	0.53±0.03	0.53±0.02	0.49±0.01	0.50±0.02	0.500
Pancreas wt. (g)	3.75±0.32	3.62±0.31	3.50±0.20	3.87±0.24	3.12±0.24	3.87±0.13	0.384
Relative pancreas wt. (LW %)	0.14±0.01	0.13±0.01	0.13±0.01	0.15±0.01	0.11±0.01	0.14±0.01	0.313
Giblets wt. (g)	92.0±3.52	92.2±4.37	97.1±5.22	96.1±3.47	94.2±1.94	90.6±5.65	0.892
Relative giblets wt. (LW %)	3.60±0.06	3.51±0.17	3.66±0.15	3.71±0.08	3.57±0.12	3.46±0.18	0.841
Abdominal fat wt. (g)	29.5±2.72	19.6±2.65	27.0±0.68	23.7±3.20	22.6±4.60	26.5±3.43	0.401
Relative abdominal fat wt. (LW %)	1.15±0.10	0.75±0.12	1.02±0.04	0.91±0.12	0.86±0.18	1.01±0.12	0.393
Gizzard wt. (g)	33.5±1.40	32.5±2.94	36.1±3.43	39.1±1.14	34.5±3.46	36.6±1.64	0.567
Relative gizzard wt. (LW %)	1.31±0.02	1.23±0.11	1.35±0.11	1.51±0.04	1.30±0.14	1.40±0.07	0.450
Duodenum wt. (g)	11.6±1.33	11.8±0.43	12.1±0.55	10.2±0.78	10.7±0.52	10.3±0.66	0.274
Relative duodenum wt. (LW %)	0.45±0.04	0.45±0.01	0.45±0.03	0.39±0.04	0.40±0.01	0.39±0.02	0.241
Jejunum wt. (g)	39.7±0.63	40.2±1.89	37.8±1.66	40.1±2.55	39.7±2.29	43.1±4.90	0.778
Relative jejunum wt. (LW %)	1.56±0.06	1.53±0.06	1.43±0.07	1.54±0.09	1.50±0.08	1.64±0.17	0.594
Ileum wt. (g)	4.87±0.31	4.62±0.47	4.75±0.72	4.12±0.31	4.62±0.63	4.62±0.55	0.847
Relative ileum wt. (LW %)	0.19±0.01	0.17±0.02	0.18±0.03	0.15±0.01	0.17±0.02	0.17±0.02	0.806
Small intestine wt. (g)	54.1±3.20	55.0±2.12	54.7±2.73	51.8±3.84	55.1±3.24	52.8±3.80	0.936
Relative small intestine wt. (LW %)	2.11±0.09	2.09±0.07	2.07±0.12	2.00±0.15	2.08±0.11	2.01±0.11	0.928

T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T_3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

Likewise, past researches reported that feeding of NLM (Ubua *et al.,* 2019; Egbeyale *et al.,* 2020), aqueous fresh NLE (Shaahu *et al.,* 2020), and OA (ELnaggar *et al.,* 2017) did not influence the yield of visceral organs in broilers. In disagreement to these results, Paul *et al.* (2020) and Abou–Ashour, *et al.* (2021) observed higher weights of different visceral organs of chickens fed on NLE and CA, respectively. In this study, the weight of lymphoid organs was comparable among the groups (Table 4) which is in accordance with the findings of Egbeyale *et al.* (2020) and Hassan *et al.* (2016) in broilers supplemented with NLM and CA, respectively. However, a higher weight of lymphoid organs was observed by Ansari *et al.* (2012) and Abou-Ashour *et al.* (2021) in broilers supplemented with NLM and CA, respectively.

Table 4. Effect on lymphoid organs weight in broilers supplemented with NLM and CA

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
Thymus wt. (g)	4.75±0.48	5.50±0.50	5.25±0.75	5.75±0.48	5.75±0.85	5.50±0.50	0.806
Relative thymus wt. (LW %)	0.19±0.01	0.21±0.01	0.20±0.02	0.22±0.02	0.22±0.03	0.22±0.02	0.902
Bursa wt. (g)	6.00±0.00	6.75±0.25	6.00±0.00	6.25±0.25	6.00±0.00	6.50±0.29	0.072
Relative bursa wt. (LW %)	0.22±0.01	0.25±0.01	0.23±0.01	0.24±0.01	0.23±0.01	0.24±0.01	0.281
Spleen wt. (g)	3.75±0.85	4.50±0.29	4.25±0.25	4.50±0.50	3.50±0.29	3.75±0.48	0.597
Relative spleen wt. (LW %)	0.14±0.03	0.17±0.01	0.15±0.01	0.16±0.01	0.13±0.01	0.13±0.02	0.504

T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T_3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

The measurement of the small intestine were found comparable in the groups (Table 5) which is supported by several researchers who reported that dietary inclusion of NLM (Ubua *et al.,* 2019; Egbeyale *et al.,* 2020), neem seed oil (Sonhafouo *et al.,* 2019) and acetic acid (AA) (Sorathia, 2016) in broilers did not influence the length of the small intestine. However, Nourmohammadi *et al.* (2010) reported increased (P<0.01) jejunal length in broilers supplemented with 6% CA.

Table 5. Effect of supplementation of NLM and CA on length (cm) of small intestine in broilers

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
Duodenum	26.5±0.95	26.8±0.72	28.6±0.92	28.5±0.84	27.3±1.42	27.8±1.01	0.452
Jejunum	109.8±3.39	112.8±1.14	106.1±3.94	110.5±5.83	111.7±3.25	111.8±1.88	0.820
Ileum	12.4±0.19	13.0±0.99	12.6±0.37	13.3±0.66	13.3±0.52	12.3±0.52	0.758
Small intestine	148.8±3.45	152.8±0.81	147.5±4.10	152.3±5.87	152.5±4.02	152.0±2.47	0.883

T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

The data presented in Table 6 revealed a significant (P<0.05) fall in caecal E. coli counts in groups T2 and T3 than T0. Similar results were found in broilers supplemented with CA (ELnaggar *et al.,* 2017), OA (Fathi *et al.,* 2016; Eftekhari *et al.,* 2015), and OA blend (Sultan *et al.* 2015). Likewise, the non-responsive impact of NLM on the E. coli population of the present study is in agreement with the findings of da Silva Assunção *et al.* (2019) and Vanessa *et al.* (2019) in broilers supplemented with NLM and neem oil, respectively. In contrast to these results, Esmaeilipour *et al.* (2012) observed a non-significant effect of OA on the E. coli population in broilers. The inhibitory effect of CA on gut E. coli might be due to the lowering of the intestinal pH through dietary acidification. Acidifiers in the feed inhibit the growth of pathogenic bacteria by altering the gut pH and hence, reducing the microbial competition for host nutrients. The proliferation of most pH-sensitive bacteria like E. coli, Salmonella, and Clostridium is minimized under acidic pH. The organic acids (undissociated form) penetrate the bacterial cell and after dissociation cause lowering of cytoplasmic pH. At lower pH, the enzymatic reactions of glycolysis signal transductions and nutrients transport to the microbes are impeded causing energy deprivation on its effort to recoup the normal pH (Mroz *et al.,* 2006). Further, the trapped anions of the acids cause toxicity and disrupt bacterial membranes (Russel, 1992).

Table 6. Caecal microbial count (log10 × 10 6 cfu/g) and pH of broilers supplemented with NLM and CA

Parameter	Dietary group						P- value
	T_0	T_1	T_2	T_3	T_4	T_5	
E. coli	11.7±1.11^b	9.75±1.92^b	7.02±0.02^a	6.75±0.14^a	9.87±0.59^b	9.75±0.63^b	0.006
Caecal pH	7.15±0.08^c	6.99±0.05^b	6.79±0.02^a	6.83±0.01^a	6.78±0.02^a	6.81±0.04^a	0.000

^{abc} values in a row bearing different superscript differ significantly (P<0.05)
T_0 (control): basal diet, T_1: basal diet + 2.5 g of NLM per kg of feed, T_2: basal diet + 15 g of CA per kg of feed, T_3: basal diet + 25 g of CA per kg of feed, T_4: basal diet + 2.5 g of NLM + 15 g of CA per kg of feed and T_5: basal diet + 2.5 g of NLM + 25 g of CA per kg of feed

In the present study, the caecal pH reduced significantly (P<0.05) in treatments T1, T2, T3, T4, and T5 than T0. Likewise, previous workers reported that dietary supplementation of CA (Nourmohammadi and Khosravinia, 2015), OA (Jadhao *et al.,* 2020), a mixture of propionic- acetic acid (Martínez *et al.,* 2021) and AA (Sorathia, 2016) had significantly reduced the caecal pH in broilers. However, contradictorily, Hassan *et al.* (2016) and Esmaeilipour *et al.* (2011) observed a non-responsive effect of CA on caecal pH in ducks and broilers, respectively. The low caecal pH in the present study could possibly be due to more synthesis of volatile fatty acids (VFA) such as acetic, butyric, and propionic acid as a consequence of anaerobic degradation (Jozefiak *et al.,* 2011). Moreover, large numbers of lactobacilli in the caeca add more VFA which further decrease the caecal pH in the poultry gut (García *et al.,* 2008).

CONCLUSION

It may be concluded that feeding of CA at 15 g and 25 g per kg of feed in broiler chickens may improve the gut health without any ill effect on carcass and visceral organs. Whereas, neither carcass characteristics nor intestinal health influenced by supplementation of either NLM or a combination of NLM+CA in broilers. Therefore, the inclusion of CA at two dietary levels i.e. 15 g and 25 g per kg of feed in broiler ration could be a better alternative to produce healthy broiler chickens.

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