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Dietary of probiotics and organic acids supplementation on productive performances, intestinal morphology, carcass characteristics, and meat quality of broiler chickens

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Abstract In theantibiotic-free and food safety era, broiler diet formulations emphasize theuse of innocuous feed additives such as probiotics, prebiotics, synbiotics, and andorganic acids. Furthermore, it is used to replace antibiotics in broilers productionin order to maintain the ecological balance of the gut, enhance intestinal morphology, increase productivity, and improve feed efficiency. Results showed that supplementation ofprobiotics and a combination of probiotics and organic acids in the level of 2kg/ton feed had significantly higher ($P<0.05$) villi height when comparedwith other treatments. It was also discovered that probiotics and organic acidssupplementation improved ($P<0.05$) feed conversion ratio of broilers in the starter period (1-21 days) in comparison to the other groups. However, therewas no effect of any of the dietary treatments on growth performance during thegrower period (22-42 days) or the finisher period (43-56 days). Likewise, probiotics, organic acids, and combinations of probiotics and organic acids had no effecton broiler carcass traits or meat quality characteristics. However, probioticsand organic acids had no effect on carcass traits in terms of increasing theproportion of breasts and lowering the percentage of abdominal fat pads ($P>0.05$).This experiment concluded that a 2 kg/ton feed supplementation of probiotics combined with organic acids improved growth performance in terms of feedconversion ratio and villi height of female broilers during the starter period.

Keywords: Probiotic, Organic acid, Diet, Feed additive, Broiler

Introduction

There is a growing concern that the effects of climate change will pose a serious challenge to sustainable broiler production. Broiler production systems will have to focus not only on increasing output but also on their environmental

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and human and animal health impacts. Heat stress events in livestock are expected to become more common as a result of the climatic challenge (Rahimi *et al*., 2020) and bird diets must be adjusted to the climate conditions. Antibiotics were used to improve the productivity of meat-producing chickens reared in tropical climates, as well as their energy utilization in particular. However, the use of antibiotics that promote animal growth is currently being investigated due to growing consumer concerns about the use of antibiotics in feed, which will result in resistance to human pathogenic bacteria. (Manyi-Loh *et al*., 2018). This condition has prompted extensive research into alternatives capable of maintaining high productivity while remaining economically viable and non-harmful to human and animal health, thereby meeting the needs of consumers and foreign markets (Bitterncourt *et al*., 2016). Gonzales *et al*. (2013) reported the positive effects of organic acids and probiotic microorganisms (Al-Khalaifa *et al*., 2019) which can be used as a safe feed additive and a substitute for antibiotics in farm animals to maintain the ecological balance of the gastrointestinal tract, promote productivity, and improve feed efficiency.

Probiotics are living microorganisms that provide health benefits to animals when properly administered and in sufficient quantities for animal use. (Krysiak *et al*., 2021). The majority of probiotics are produced by bacteria such as *Bifidobacterium* spp., *Lactococcus* spp., *Lactobacillus* spp., *Bacillus* spp., *Streptococcus* spp., and yeasts such as *Saccharomyces* spp. (Park *et al*., 2016). Probiotic bacteria that have been isolated are engineered to produce enzymes or substances that stimulate the activity of phytase, cellulase, protease, or xylanase (Krysiak *et al*., 2021). However, probiotic colonization in the gut is dependent on a variety of factors, including the availability of fermentation prebiotics (Rehman *et al*., 2020). The usage of probiotics encourages the growth of beneficial microorganisms rather than potentially pathogenic bacteria. Probiotics have a competitive exclusion mechanism, which allows them to prevent pathogenic bacteria like *Clostridium perfringens* and *Salmonella* spp. from colonizing the gut. (Murshed and Abudabos, 2016). De Souzaa *et al*. (2018) reported probiotics are effective in developing the intestinal mucosa, increasing surface area for nutrient absorption, increasing growth rate, and having a significant impact on carcass yields, cutting products, meat quality, and promoting immune responses (Soomro *et al*., 2019). Bacillus-based probiotics have several advantages, including temperature tolerance, ease of cultivation, and low production costs. *Bacillus*' ability to withstand temperature changes is required at certain stages of feed processing. Current knowledge contributes to an understanding of the role of gut microbes in immune system development and increases the expression of cytokinins and interleukins (Yu *et al*., 2020).

The addition of organic acids to the diet to normalize the gastrointestinal tract with weak organic acids such as lactic, propionic, lactic, pyroligneous acid or wood vinegar, formic, fumaric, and sorbic acids has a clear effect on helping reduce germ colonization and the production of toxic metabolites as well as improves crude protein digestion and mineral retention, and acts as a precursor in the medium's metabolic process (Hassan *et al*., 2010). Organic acids are known to contribute to a variety of factors, including reducing diet buffering capacity, lowering the pH of the stomach and intestines aids in the regulation of the microflora in the digestive and respiratory organs, improving immune responses in poultry by increasing nutrient availability, absorption, and digestion (Nguyen *et al*., 2018). The review of the literature showed that organic acids preserve the cellular integrity of the gut lining and improve digestion by preserving normal gut flora (Sabour *et al*., 2019). In addition, supplementing organic acids in the diet has been shown to help improve immune responses (Zhang *et al*., 2011), regulate the proper pH level in the intestines (Upadhaya *et al*., 2014), as well as improve nutrient digestibility, feed utilization, (Ao *et al*., 2009), and productivity in broiler chickens (Panda *et al*., 2009). Furthermore, Organic acids can kill bacteria by entering cell lipid membranes and lowering intracellular pH, as well as stimulate the pancreatic secretion and improve intestinal villus integrity (Fascina *et al*., 2012).

However, there is still limited information on the influence of blending two commercial feed additives of the probiotic and organic acid mixture in poultry chicken. Therefore, the purpose of the investigation was to evaluate the effects of probiotic and organic acid supplementation in broiler chicken diets reared in tropical climates on growth performance, intestinal morphology, carcass characteristic, and meat quality.

Materials and methods

Experimental design, animal and diets

A total of 1,440 one-day-old mixed-sex broiler chicks (Ross 308®) were distributed in a completely randomized design with 4 treatments and 5 replicates of 72 chicks each (36 females and 36 males) and allocated floor pens with new rice hulls in an open-sided house system. The treatments were: control diets (Treatment 1), control diets plus 2 g/kg of organic acids (Treatment 2), control diets plus 2 g/kg of probiotics (Treatment 3), and control diets plus 2 g/kg of organic acids + 2 g/kg of probiotics (Treatment 4).

Table 1. Ingredients and nutrient composition of experimental diets

Items	Starter (1 to 21 d)	Grower (22 - 42 d)	Finisher (43 - 56 d)
Ingredient, (% as fed basis)			
Corn	51.00	53.00	55.00
Rice bran	10.00	10.00	10.00
Soybean meal, (48% CP)	26.46	24.36	21.50
Fish meal, (58% CP)	6.00	6.00	6.00
Palm oil	3.63	4.16	5.00
Dicalcium phosphate	0.95	0.69	0.69
Limestone	0.93	0.81	0.81
Sodium chloride	0.35	0.35	0.35
Minerrals	0.25	0.25	0.25
Vitamins	0.25	0.25	0.25
DL-Methionine	0.18	0.14	0.15
Total	100	100	100
Calculated composition, %			
ME, kcal/kg	2,975	3,050	3,100
Protein	21.5	20.00	19.50
Calcium	1.00	0.90	0.90
Non-phytic phosphorus	0.45	0.40	0.40
Lysine	1.15	1.07	1.00
Methionine	0.55	0.50	0.50
Threonine	0.75	0.70	0.65
Tryptophan	0.20	0.18	0.16
Price (bahts/kg) (October, 2016)	17.65	17.34	17.45

This experiment used dry probiotics containing 5×10^{11} CFU/kg of *Bacillus* count NLT, 620.00 g/kg of dried fermentation products, and nutritive media, 347.00 g/kg of sodium sulfate, and 3.0 g/kg of anti-caking agent. Furthermore, organic acids consist of 320 g/kg fumaric acid, 30 g/kg formic acid, 130 g/kg lactic acid, 30 g/kg propionic acid, and 10 g/kg citric acid were likewise used.

Growth performance measurements

Nutrient composition of basal diets was designed to meet the needs of broiler chickens raised in tropical climates by the National Research Council, (1994) and Table 1 shows the feed formulation and calculated nutrient composition of diets. All birds had *ad libitum* access to diets and water in three periods: starter (1 to 21 days), grower (22 to 42 days), and finisher (43 to 56 days). The number of birds, feed consumption, and weighed initial and final weights of broilers in each unit to calculate body weight gain (BWG), average daily gain (ADG), feed intake (FI), feed conversion ratio (FCR), and mortality rate for each period were recorded using the method described by Marcu *et al*. (2013), and feed cost per gain (FCG) using the method described by Nopparatmaitree *et al*. (2013).

Intestinal morphology carcass characteristic, and meat quality measurements

On a final day, 4 birds per pen (2 females and 2 males) were randomly selected, weighed, and euthanatized by severing the jugular vein. Tissue samples were collected from the middle of the duodenum and washed with a 0.9 percent saline solution. Samples were fixed in a 10% formaldehyde-phosphate buffer and stored at 4℃ for microscopic examination of intestinal morphology (Li *et al*., 2012). Sample slides were prepared for microscopic analysis slides according to Shang *et al*. (2016). Small intestine histomorphology was measured and analyzed according to Tsirtsikos *et al*. (2012) and Sakamoto *et al*. (2000). In addition, 6 birds per pen (3 females and 3 males) were randomly selected for processing. After 6 hours of feed withdrawal, the birds were weighed, electrically stunned, and manually eviscerated. Carcasses, cutting parts, and abdominal fat was weighed and recorded to calculate carcass and cutting percentage according to Faria *et al*. (2010). Meat quality in breast muscle with pH was measured by a pH meter; meat color by Hunter lab color meter; water holding capacity and shear force by warner Blatzler shear devices by Barbosa *et al*. (2017) and Pathare *et al*. (2013).

Statistical analysis

Data used the analysis of variance (ANOVA) and the difference between treatment means was compared using Duncan's new multiple range test according to Steel and Torrie (1992) by R program version 3.5.1 (R core team, 2018), which was significant at $P<0.05$ and $P<0.01$.

Results

Intestinal morphology development

The effect of supplementation of probiotic and organic acid on intestinal morphology development of broilers are presented in Table 2. The supplementation of probiotics and probiotics with organic acids resulted in a significant increase in female broiler duodenal height ($P<0.05$), but had no effect on male broiler duodenal height, crypt depth, and height-depth ratio of male and female duodenal villus ($P>0.05$).

Table 2. Effect of probiotic and organic acid supplementation in broiler diets on duodenum histomorphology[1/]

Duodenum histomorphology	Type and level of feed additive in diets: Control	Organic acid 2 kg/ton	Probiotic 2 kg/ton	Organic acid 2 kg/ton + Probiotic 2 kg/ton	SEM
Male					
Villus height (µm)	587.76	597.36	569.66	511.89	19.14
Cryptal depth (µm)	86.37	85.78	98.97	75.92	4.72
VCR[2/]	7.10	7.08	5.77	6.86	0.32
Female					
Villus height (µm)	561.42[ab]	482.64[b]	622.62[a]	609.50[ab]	31.66
Cryptal depth (µm)	83.96	84.37	90.32	88.76	1.59
VCR[2/]	6.82	5.74	6.88	6.98	0.29

[a-b] Means from columns with no common superscript differ significantly (P<0.05)
[1/] Means represent 5 replicates with 72 chicks per replicate for each treatment.
[2/] VCR=Villus height/crypt depth ratio of duodenum of female

Production performances

The average feed intake, body weight gain, and feed conversion ratio of broilers as fed with probiotic and organic acid are presented in Table 3. There was no significant difference for broiler fed with a diet supplemented with probiotic and organic acid during 1-21, 22-42, 43-56, and 1-56 days of age on production performances. However, it was found that supplementation of probiotics combined with an organic acid in the levels of 2 kg per ton feed had significantly improved (P<0.05) feed conversion ratio of broilers during 1-21 days of age. It was also discovered that supplementing probiotics at a rate of 2 kg per ton feed significantly reduced (P<0.05) feed cost per kg body weight of broilers during 43-56 days of age.

The overall production performance over the 0-56 days period was not statistically different in all observed values, which was consistent with several previous trials that reported Chick growth performance was unaffected by organic acids. Acidifiers had a tendency to improve body weight gain and feed intake (P>0.05), but these answers were inadequate to cause a statistically significant effect on average daily gain and finishing body weight.

Table 3. Effect of probiotic and organic acid supplementation in broiler diets on growth performance[1/]

Growth performances	Control	Type and level of feed additive in diets: Organic acid 2 kg/ton	Probiotic 2 kg/ton	Organic acid 2 kg/ton + Probiotic 2 kg/ton	SEM
0 to 21 days of age					
BWG (g/b)	778.40	779.11	755.66	776.63	5.62
FI (g/b)	1247.16	1250.01	1245.94	1224.82	5.78
FCR	1.61[a]	1.61[a]	1.65[a]	1.58[b]	0.02
FCG (bahts/kg)	28.96	29.08	30.06	28.82	0.28
Mortality rate (%)	2.71	1.35	2.71	4.06	0.55
22 to 42 days of age					
BWG (g/b)	1225.50	1219.11	1217.48	1216.88	1.98
FI (g/b)	1705.33	1701.59	1699.84	1722.05	5.08
FCR	1.39	1.40	1.40	1.41	0.00
FCG (bahts/kg)	24.78	24.86	25.04	25.43	0.14
Mortality rate (%)	4.06	5.19	3.27	6.77	0.76
42 to 56 days of age					
BWG (g/b)	1289.20	1250.20	1353.80	1321.40	22.17
FI (g/b)	3783.20	3770.00	3731.20	3814.00	17.15
FCR	2.94	3.03	2.76	2.94	0.06
FCG (bahts/kg)	52.45[ab]	54.15[a]	49.80[b]	52.39[ab]	0.90
Mortality rate (%)	5.58	6.95	3.61	8.92	1.12
0 to 56 days of age.					
BWG (g/b)	3293.10	3248.42	3326.94	3314.92	17.29
FI (g/b)	6735.69	6721.60	6676.98	6760.87	17.59
FCR	2.05	2.07	2.01	2.04	0.01
Mortality rate (%)	6.67	8.62	5.00	6.25	0.75

[a-b] Means from columns with no common superscript differ significantly ($P<0.05$)
[1/] Means represent 5 replicates with 72 chicks per replicate for each treatment.

Carcass characteristic and meat quality

The effect of supplementation of probiotics and organic acid on carcass characteristics and meat quality of broilers are presented in Table 4. The results showed that probiotic and probiotic plus organic acid supplementation had no significant effect on carcass percentage (including a percentage of the thigh, wing, and gizzard) and meat quality (including the percentage of pH and color of breast meat). However, it was also found that supplementation of probiotic and probiotic plus organic acid has a tendency to increase eviscerated carcass, breast, and drumstick percentage, and tended to reduce abdominal fat pad ($P>0.05$).

Table 4. Effect of probiotic and organic acid supplementation in broiler diets on carcass trait and meat quality[1/]

Carcass trait and meat quality	Control	Type and level of feed additive in diets: Organic acid 2 kg/ton	Probiotic 2 kg/ton	Organic acid 2 kg/ton + Probiotic 2 kg/ton	SEM
Carcass percentage (%)					
Dressing percentage	72.89	72.85	72.73	73.01	0.06
Pectoralis major	26.36	26.47	26.86	26.71	0.11
Pectoralis minor	12.72	12.37	12.93	12.62	0.12
Thigh	23.91	21.86	22.07	22.01	0.48
Drumsticks	20.64	20.80	20.96	20.72	0.07
Abdominal fat	6.24	5.52	5.77	5.78	0.15
Meat quality					
pH	6.12	6.15	6.13	6.23	0.12
Meat Colors					
Ligthness (L^*)	50.58	53.46	52.20	53.08	0.64
Redness (a^*)	5.63	4.25	5.08	4.51	0.31
Yellowness (b^*)	19.03	17.84	18.08	19.38	0.37
Water holding capacity (%)					
Drip loss	2.62	2.12	2.91	2.28	0.18
Cooking loss	13.47	14.34	14.21	13.04	0.31
Shear force	1.60	1.40	1.33	1.36	0.06

[1/] Means represent 5 replicates with 72 chicks per replicate for each treatment.

Discussion

There are plausible explanations for the contribution of organic matter to the development of the intestinal mucosa about bacteria in the gut secrete a variety of harmful compounds that reduce fat digestibility, stimulate speedy turnover of absorptive epithelial cells, and stimulate mucus secretion. These factors slow growth performance, and microflora is responsible for approximately 6% of net energy losses in animals (Suiryanrayna *et al*., 2015). Organic acids have the ability to eliminate specific species such as *Coliforms* while causing aerobiosis. As a result, they can provide an optimal microbial environment in the gut, benefiting the host by accumulating fewer toxic compounds, amines, and ammonia (Rathnayake *et al*., 2021). Organic acids have the ability to easy time penetrate the bacteria cell wall as well as interfere with normal cellular functions such as replication and protein synthesis (Nguyen, *et al*., 2020). The proposed consecutive mechanisms of a bactericidal act are as follows: organic acids in their acid form (protonated form) can pass through the bacteria cell wall, within bacterial cells, penetrated organic acids dissociate into the conjugated base form (non-protonated form), lowering cellular pH and creating a stressful environment, This causes cell dysfunction and, as a result, prevents bacterial growth. Such reactions are most commonly seen in pH-sensitive bacteria or pathogenic bacteria (Mani-Lopez *et al*., 2012). Akyurek *et al*. (2011) reported that the addition of organic acids to broiler diets resulted in a lower number of pathogenic bacteria, such as *Coliform* and *Clostridia*, than those fed AGP diets. However, it can aid in the growth of beneficial bacteria in the ileum, such as *Lactobacillus*. It is also possible that the decreased pH in the gut caused by dietary organic acids helps to prevent the bacterial transfer from the diet and atmosphere. Moreover, short-chain fatty acids (SCFAs) are byproducts of cecum microbial fermentation. They play an important role in a variety of situations, including energy metabolism, gastrointestinal performance, and pathogen reduction in fowls (Meimandipour *et al*., 2010). SCFA concentrations have been linked to intestinal microbial colonization. As a result, it is critical to recognize the ability of lactobacillus and bacillus in different colonization in the intestines. Although *Lactobacillus* and *Enterococcus* spp. colonize the gut, *Bacillus* spp. are free-flowing and do not colonize it (Huyghebaert *et al*., 2011). SCFA not only provides energy to cell membranes but also have an indirect effect on lowering caecum pH, which inhibits pathogen growth and increases mineral uptake (Pourabedin and Zhao, 2015). Butyric acid is the epithelial cell's primary source of energy, and it also aids in the suppression of the inflammatory response by inhibiting proinflammatory cytokines (Eeckhaut *et al*., 2011). These findings support the notion that dietary probiotic supplementation increases villous height. For example, Samanya and Yamauchi (2002) reported that broiler feeding with a *Bacillus subtilis* supplemented diet resulted in a significant increase in villus height in the duodenum and ileum of 28-day-old chickens. Awad *et al*. (2008) discovered that broilers fed *Saccharomyces cerevisiae* for the first seven days had higher villous height than the control group. These findings are most likely the result of a rise in SCFA levels in the gastrointestinal tract caused by probiotic supplementation in feed. Furthermore, in vitro studies on the ability of probiotics to increase short-chain fatty acid levels while decreasing ammonium production have been published (Rahimi *et al*., 2009).

This experiment demonstrated that organic and probiotic supplementation improved broiler feed efficiency during the starter period of rearing when the gastrointestinal tract function and digestive enzyme secretion were developing. These findings are consistent with those of Nourmohammadi *et al*. (2011) who found a significant pH decrease in the duodenum, while other studies discovered no significant pH decreases. The jejunum, ileum, and cecum all had similar pH-lowering tendencies. Organic acids are easily absorbed in the upper gastrointestinal tract, and only organic acids added to diets can reach the lower gastrointestinal tract (Kim *et al*., 2015). Lowering the pH in the upper gut may improve nutrient digestion and lead to increased nutrient utilization in diets because the enzyme pepsinogen and other zymogens in the stomach are stimulated by lower gastric pH (Nguyen *et al*., 2020). It may be due to organic acid had the activity like acidifiers which increased gastric proteolysis and protein-amino are digestible by increasing the activity of digestive enzymes (Salgado-Transito *et al*., 2011). This increased enzyme activity has the potential to increase protein digestion and possibly other nutrient digestion. Furthermore, Acidic digesta may slow gastric emptying, giving the GIT more time to digest nutrients (Kim *et al*., 2015). Mellor (2000) observed that adding organic acids to broiler diets resulted in a change in intestinal pH, which helped to promote the function of probiotics and maintain a healthy microbial environment in the bird's gastrointestinal tract, as well as reduce the number of pathogenic microorganisms. These phenomena result in increased digestion, absorption, and feed utilization efficiency. (Fritts *et al*., 2000). In addition, Awad *et al*. (2008) and Rahimi *et al*. (2009) both studies found that supplementing with probiotics and synbiotics can improve gut morphology in terms of villus height, which is directly proportional to the increase in epithelial area used for digestion and absorption of nutrients. This has a direct effect on the increase in mucosal enzymes, uptake, and transport nutrient systems.

It was also found that supplementation of probiotics and probiotics plus organic acid did not significantly improve meat quality in terms of drip loss, cooking loss, and shear force. The results of this experiment were in the same direction as the results of Midilli *et al*. (2008) who used 0.05 percent probiotics (*Bacillus licheniformis* and *Bacillus subtilis*) feed to broiler chickens for 42 days and found no significant improvements in carcass weight, or carcass yield. Furthermore, Kopecký *et al*. (2012) found that organic acid supplemented in drinking water had no effect on carcass yield, percentage of breast and thighs, or average abdominal fat weight. In terms of live body weight, net carcass, dressing percentage, and comparative weight of the visceral organ, the addition of *Bacillus subtilis*, acidifiers, and their combination had no significant effect (Huwaida *et al*., 2016).

Based on the finding, it was concluded that supplementation of probiotic and probiotic plus organic acid at 2 kg/ton feed significantly improved broiler feed conversion ratio in starters (1-21 days of age) and also improved intestinal duodenum villus height of female broilers. Likewise, had no effect on carcass percentage in terms of breast percentage; however, it did tend to reduce broiler abdominal fat pad; as well as non-significant improvement in meat quality in terms of drip loss, cooking loss, and shear force of broiler breast muscle.

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