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Effects of Mannanase and Citric Acid Supplementation on the Growth Performance of Broilers

Maria Patricia Arellano[1], Listya Purnamasari[2], and Joseph dela Cruz[1]*

[1]Department of Basic Veterinary Sciences College of Veterinary Medicine, University of the Philippines Los Baños Laguna, 4031, Philippines
[2]Department of Animal Husbandry, Faculty of Agricultural, University of Jember, Jember, 68121, Indonesia

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* Corresponding author:
Telp. +63 93 6936 1601
E-mail: jfdelacruz@up.edu.ph

ABSTRACT

The study was conducted to evaluate the effects of citric acid and mannanase feed supplementation on the growth performance of broiler chickens. Three hundred twenty day-old unsexed Cobb broiler chicks from a commercial hatchery plant were used in the study. The chicks were randomly assigned to four treatment groups following a completely randomized design. The following treatment groups were used: T1: Control, T2: 3% (w/w) Citric Acid supplementation, T3: 80 ppm β-Mannanase supplementation, and T4: 3% (w/w) Citric Acid and 80 ppm β-Mannanase supplementation. The supplementation of citric acid and mannanase significantly improves the growth performance of broiler chickens. Body weight and body weight gain were significantly higher ($P<0.05$) in the citric acid and β-mannanase combination group compared to all the treatment groups. The evaluation of feed intake revealed that T3 and T4 had significantly lower ($P<0.05$) feed intake than the other groups. The combination of citric acid and β-Mannanase also resulted in a significantly more efficient ($P<0.05$) feed conversion ratio. No mortality was observed during the experiment. It may be concluded that the supplementation of citric acid and β-mannanase resulted in a better performance in broilers.

Keywords: Broiler, Citric acid, Feed additive, Growth performance, β-Mannanase

Introduction

Broiler production is a progressive industry and a big contributor to the agriculture sector of the Philippines. According to the Philippine Statistics Authority, the total inventory of chickens as of July 1, 2019, has reached 191.70 million birds. Broiler chicken inventory grew from 64.94 million birds in 2018 to 68.97 million birds in 2019, which amounts to a growth of 6.2%. Animal production involves the administration of antibiotics to prevent and/or treat diseases and as growth promoters. It has been an integral part of the poultry industry for many decades (Alloui, 2014).

The inclusion of antibiotics in feed as a growth promoter is beneficial in disease prevention and improvement of production parameters, ultimately leading to better profit. The indiscriminate use of antibiotics in the poultry industry can result to the deposition of residues in products such as meat and eggs, which poses a potential threat to public health (Yang *et al.,* 2020). Currently, the subtherapeutic level of antibiotics is still widely used for growth promotion (Khalique *et al.,* 2020). The demand for growth promoters emerged because of the presence of anti-nutritional factors in feed. These substances negatively affect growth performance by inhibiting nutrient absorption and weakening the immune system (Zheng *et al.,* 2017). Organic acids and exogenous enzymes are used as alternative feed additives that also promote growth through maximizing nutrient utilization without the risk of antibiotic residues (Dittoe *et al.,* 2018; Cowieson and Kluenter, 2019).

Organic acids like citric acid are widely added to drinking water for sanitation and in feed to prevent degradation caused by microorganisms and fungi (Hassan *et al.,* 2012). Acidification of feed creates an environment in the digestive tract that is favorable for utilizing nutrients such as proteins (Nourmohammadi *et al.,* 2010). Exogenous enzymes are added to the diet of broilers to improve the utilization of nutrients from the feed by correcting the deficiency in endogenous enzymes or hydrolyzing anti-nutritional factors (Mustafa and Jameel, 2013). Enzymatic activity is prominently affected by the pH of the digest. According to Abdollahi *et al.* (2022), feed acidification improves the efficacy of exogenous enzymes by lowering the pH of the diet and digesta. There are recent studies on the positive effects of the separate supplementation of citric acid (Abd-Elsamee, 2020) and β-Mannanase (Mohammadigheisar *et al.,* 2021). One unit of mannanase activity was defined as the amount of enzyme which generates 0.72 micrograms of reducing sugars per minute from a mannose-containing substrate at pH 6.6 and a temperature of 104°F (Caldas *et al.,* 2018).

In addition, there have been studies on the simultaneous supplementation of other organic acids like phytic acid and enzymes like phytase (Cowieson *et al.,* 2010). However, at present, there are no studies on the combined effects of citric acid and mannanase on the growth performance of broilers. The aim of this study was conducted to evaluate the effects of citric acid and mannanase feed supplementation on the growth performance of broiler chickens.

Materials and Methods

A total number of 320 day-old unsexed Cobb broiler strain chicks from a commercial hatchery plant was used in the study. The experimental protocol was approved by the University Institutional Animal Care and Utilization Committee. The chicks were allocated into 4 treatment groups (T1-T4) following a completely randomized design (CRD). Each treatment was replicated eight times with 10 chicks per replicate. The corn-soybean-based diets were formulated according to the nutritional requirements for starter, and finisher rations and was presented in Table 1. Feeds used in the study were free from antimicrobial agents. Inclusion rates of citric acid and β-Mannanase were recommended by the manufacturer (Henan New Yangshao Biotechnology Co., Ltd).

The following treatment groups were used: T1 (control): Basal feed without organic acid and enzyme supplementation; T2: Basal diet plus 3% (w/w) Citric Acid; T3: Basal diet plus 80 ppm β-Mannanase; T4: Basal diet plus 3% (w/w) Citric Acid and 80 ppm β-Mannanase.

Upon arrival, the average initial weight (kg) was recorded per replicate per treatment group. The growth performance was monitored per growth period. Mortality was monitored daily. Live weight (kg), livability (%), feed intake (kg), weight gain (kg), and feed conversion ratio were recorded and computed at the end of each period.

Data were analyzed using the General Linear Model with P < 0.05. The collected data were subjected to Analysis of Variance (ANOVA). Means of the treatment groups were compared using Tukey's Honest Significant Difference Test.

Results and Discussion

The initial weights ranging from 40.48 to 40.53 g did not vary significantly (P<0.05). Statistical analysis showed that treatment groups supplemented with 80 ppm β-Mannanase (T3) and 3% (w/w) Citric Acid with 80 ppm β-Mannanase (T4) were significantly higher than the control group (T1). At day 35, average body weights ranged from 1695.88 to 1810.8 g with broilers under T4 weighing significantly higher than T1, T2, and T3.

The mean feed intake did not vary significantly from days 0-10, 11-20, and 21-35 (P < 0.05). Data gathered from day 0 to day 35 showed that T4 exhibited significant differences from the average feed intake of T1 and T2. There was no significant difference between T4 and T3, but broilers under T4 consumed the least amount of feed among all treatment groups.

Weight gain of broilers in T3 and T4 was significantly higher than the control group (T1) from days 0-10. At days 11-20, Treatment 2, 3, and 4 yielded significantly higher weight gain than the control group. The weight gain during this growth period ranged from 607.75 to 634.88 g. Data gathered at days 21-35 showed that treatments 3 and 4 had significantly higher weight gain than the control group and treatment 2. During the whole duration of the study, the weight gain of broilers in Treatment 4 significantly yielded the highest gain compared to the other treatment groups.

The average feed conversion ratio of broilers in all stages of production is presented in Table 2. On days 0-10, T4 showed a more efficient FCR compared to the control group. During the starter period (days 11-20), treatments 2, 3, and 4 performed with a better FCR than the control group. Data analysis for days 0 to 35

Table 1. Nutrient composition (%) of broiler corn-soybean-based diets used in the experiment

Item	Starter diets	Finisher diets
Ingredients (%)		
Corn	61.55	66.54
Soybean meal (CP = 47,8%)	33.41	29.26
Soybean oil	0.80	0.69
Dicalcium phosphate	1.77	1.26
Limestone	1.32	1.38
Sodium chloride	0.38	0.25
DL- methionine	0.22	0.07
Premix	0.5	0.5
Choline chloride	0.05	0.05
Analyses		
ME (kcal/kg)	2950	3000
Crude protein (%)	21.47	19.89
Lysine (%)	1.15	1.04
Methionine+cystine (%)	0.90	0.72
Dry matter (%)	89.29	89.32

Table 2. Effect of citric acid and β-Mannanase supplementation on the performance of broilers

	T1	T2	T3	T4
Body weight (g)				
Day 0	40.50±0.24	40.53±0.40	40.44±0.25	40.48±0.33
Day 10	302.13±5.14^{b}	307.75±5.12^{ab}	311.75±5.12^{a}	313.88±8.17^{a}
Day 21	909.88±3.48^{c}	936.63±6.84^{b}	944.38±11.21^{ab}	948.75±10.08^{a}
Day 35	1695.88±8.24^{d}	1732.60±7.63^{c}	1779.00±18.58^{b}	1810.80±17.02^{a}
Feed Intake (g)				
Day 0-10	304.63±11.71	303.50±4.54	302.38±3.99	303.25±9.65
Day 11-20	974.75±8.68	982.13±6.90	976.38±7.13	974.00±7.41
Day 21-35	1975.38±8.53	1974.63±5.97	1959.38±17.50	1958.63±25.30
Day 0-35	3254.75±14.69^{a}	3260.25±4.74^{a}	3238.13±21.77^{b}	3235.88±27.90^{b}
Weight gain (g)				
Day 0-10	261.63±5.12^{b}	267.23±5.23^{ab}	271.31±5.08^{a}	273.4±8.25^{a}
Day 11-20	607.75±7.23^{b}	628.88±10.2^{a}	632.63±14.31^{a}	634.88±11.95^{a}
Day 21-35	786.00±10.06^{c}	796.00±10.78^{c}	834.63±26.61^{b}	862.00±23.57^{a}
Day 0-35	1655.38±8.28^{d}	1692.10±7.63^{c}	1738.56±18.56^{b}	1770.28±16.99^{a}
FCR				
Day 0-10	1.17±0.05^{a}	1.14±0.03^{ab}	1.12±0.04^{ab}	1.11±0.04^{b}
Day 11-20	1.60±0.02^{a}	1.56±0.03^{b}	1.54±0.04^{b}	1.53±0.03^{b}
Day 21-35	2.51±0.03^{a}	2.48±0.03^{a}	2.35±0.08^{b}	2.27±0.05^{c}
Day 0-35	1.97±0.01^{a}	1.93±0.01^{b}	1.86±0.02^{c}	1.83±0.02^{d}
Mortality				
Day 0-35	0	0	0	0

[a,b] Different superscripts at the same row indicate significant differences (P<0.05).
T1 (control): Basal feed without organic acid and enzyme supplementation; T2: Basal diet plus 3% (w/w) Citric Acid; T3: Basal diet plus 80 ppm β-Mannanase; T4: Basal diet plus 3% (w/w) Citric Acid and 80 ppm β-Mannanase.

revealed that T4 yielded the most efficient FCR compared to other treatment groups.

Mortalities were recorded daily to assess the treatments on broiler livability. No mortalities were observed throughout the experiment as presented in Table 2. All the treatment groups have 100% survivability since the number of broilers was the same from start until the end of the study.

Poultry feeds include substances that inhibit the digestibility of nutrients called anti-nutritional factors (Zheng *et al.,* 2017). These substances include non-starch polysaccharides, specifically mannans, that reduce nutrient digestibility in broilers. Mannans can be found in soybean meals, which is one of the most important protein sources in feed ingredients. Aside from reducing digestibility, mannans also cause high intestinal viscosity that weakens the immune system and encourages microbial growth in the gut (Saeed *et al.,* 2019). Feed additives' primary objective is to enhance livestock's growth performance by increasing nutrient digestibility. The exogenous enzyme, β-Mannanase, reduces the level of mannans in the digestive tract. Acidifying the feed through the supplementation of organic acids like citric acid maximizes the activity of exogenous enzymes like β-Mannanase.

There are currently no known studies on the synergistic effects of citric acid and β-Mannanase on broiler feed as they are usually supplemented separately. However, a study conducted by Woyengo *et al.* (2010) revealed that feed supplementation of both citric acid and phytase resulted in increased digestibility. Citric acid increases the digestibility of protein and amino acids by promoting gastric proteolysis (Sultan *et al.,* 2015). Nutrients are more utilized, which would then result in improved growth and feed efficiency. A study by Cho and Kim (2013) on the effects of β-Mannanase feed supplementation on the growth performance of broilers revealed that the enzyme can enhance body weight gain, feed conversion ratio, digestibility, and meat weight. It was also found that lower energy is required in feeds supplemented with β-Mannanase, which would eventually result in lower feed cost.

In this study, the most efficient treatment was the combination of 3% citric acid and 80 ppm β-Mannanase. The broiler chickens supplemented by both citric acid and β-Mannanase resulted in the most desirable FCR and highest live weight

Broilers under treatment 4 were the heaviest at the end of the experiment (Table 1). Our finding agrees with Dehghani-Tafti and Jahanian (2016), it was concluded that the average daily gain (ADG) was increased in broilers supplemented with citric acid. According to Rafacz-Livingston *et al.* (2005), citric acid improves the utilization of phytate phosphorus, which is a mineral that is often excreted in exorbitant amounts. Citric acid acidifies the gastrointestinal tract, therefore improving the digestibility of nutrients like phosphorus. Supplementation of β-Mannanase also has a positive effect on the body weight of broilers during the starter period, according to a study conducted by Kong *et al.* (2011). It was concluded in the study that supplementing with β-Mannanase improves the gain and energy utilization of broilers. Similarly, breast meat weight was relatively higher in broilers supplemented with β-Mannanase (Cho and Kim, 2013). The β-Mannanase had an optimum condition at 70°C and pH 6 (Ariandi *et al.,* 2015).

Feed intake was not significantly affected by the treatments also found in a study on organic acids supplementation by Adil *et al.* (2010). According to the study, the strong taste of organic acids could have resulted in a decrease in the palatability of feed, which is an important factor for feed intake. On the contrary, feed consumption was observed to increase along with body weight in a study on the effects of citric acid on phytate phosphorus utilization (Rafacz-Livingston *et al.,* 2005). Similar results were also reported by Nezhad *et al.* (2007) where the supplementation of citric acid in feed did not significantly affect feed intake in broilers. However, increased feed intake was reported in CA-fed broilers by Islam *et al.* (2008) and Sharifuzzaman *et al.* (2020). Similar findings were observed in a study by Chowdhury *et al.* (2009). Increased feed intake was also observed in a study on β-Mannanase by Barros *et al.* (2015) and Jackson *et al.* (2004). On the contrary, no significant difference in feed intake in broilers fed with basal diet with β-Mannanase was reported by da Rocha *et al.* (2010).

The nutrient composition was the same in all treatments (basal diet) shown in table 1. Many fibrous ingredients in poultry feed have low digestibility and cause poor growth in poultry that reducing production costs (Singh and Kim, 2021). Fiber can act as an antinutrient and have negative effects on the viscosity of digesta, and mineral absorption through the chelating properties of some fiber parts (Bederska-Łojewska *et al.,* 2017). Enzyme supplementation to increase the digestibility of fiber will also improve the utilization of other nutrients in feed. Administration of organic acids and exogenous enzymes can obtain the additional benefits of maintenance of intestinal villi structure and increasing the production of short-chain fatty acids (SCFA) in the gut. SCFA reduce intestinal pH and limit the growth of acid-sensitive pathogenic bacteria such as Enterobacteriaceae by depleting their H+ATPase pump and increasing energy absorption of undigested nutrients (van der Wielen *et al.*, 2000; Shyer *et al.,* 2013)

FCR was found to be most efficient in broilers under treatment 4 at the end of the experiment. Better FCR was also observed by Islam *et al.* (2008), wherein the effects of citric acid and acetic acid on the growth performance of broilers were tested. Similarly, in a study conducted by El-Masry *et al.* (2017), there was a significant improvement in the FCR of broilers given feed supplemented with β-Mannanase. Improvement in the FCR was also reported by Zangiabadi and Torki (2010). The improvement in growth parameters such as FCR can be caused by the breakdown of anti-nutritional factors like mannans. Increased breakdown of this component results in increased energy availability, reduced intestinal viscosity, improved absorption of nutrients and enhanced growth performance.

There were no mortalities throughout the experiment. This indicates that the treatments do not influence the livability of broilers. According to the study of Kopecky *et al.* (2012), citric acid and acetic acid positively affect broilers' mortality. There were no deaths in broilers supplemented with the organic acid contrary to the control group. In a study on the effects of β-Mannanase on broiler performance by Mehri (2010), there is no significant effect of the enzyme on mortality. Numerically, results show that there was mortality in the control group, while there were none among the broilers fed with the highest amount of β-Mannanase in the study.

Currently, there are no studies on the effects of simultaneously supplementing citric acid and β-Mannanase in broiler feed. However, the results of this study revealed that the most desirable growth performance was observed in the group treated with both citric acid and β-Mannanase. The significant positive effects of the combination of the two additives were evident in the body weight, body weight gain and FCR. Treatment 4 resulted in a significantly higher weight gain and better FCR in comparison to all the treatment groups. The significantly enhanced growth performance can be attributed to the acidification of the gut and ingesta by citric acid, which then enables an increase in the enzymatic activity of β-Mannanase. As a result, higher levels of anti-nutritional factors like mannans are hydrolyzed; therefore, increasing nutrient digestibility and consequently enhancing growth performance.

Conclusions

In conclusion, this study demonstrated that the combination of citric acid and β-Mannanase supplementation enhanced the growth performance of broilers.

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