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Effects of supplementing diets with an acidifier on performance parameters and visceral organ weights of broilers

Hedayati M.*[1], M. Manafi[1], M. Yari[1] and P. Vafaei[2]

[1]*Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran*
[2]*Tehran Dane Feed Manufacturing Company Ltd., Tehran, Iran*

ABSTRACT

The aim of this study was to investigate the effects of the dietary supplementation of an acidifier agent on performance parameters and visceral organ weights of broilers. Two hundred and forty day old Ross 308 broilers where divided in four groups with three replicates and twenty chicks each. The present study assessed the different dosage of an acidifier on commercial broilers. Diets prepared without additive Control (CON) (group 1), 0.025% Acidifier Agent (AA1) (group 2); 0.05% Acidifier Agent (AA2) (group 3) and 0.1% Acidifier Agent (AA3) (group 4). Results showed that among weekly body weight, feed intake and FCR, there was a significant (P<0.05) improvement, however, the maximum difference were noticed in the level of 0.1%, followed by 0.05% and 0.025% levels. The minimums survivability percentage was recorded in 0.1% level. The visceral organ weights (g/kg live weight) of thymus, bursa of Fabreciuos, liver and pancreas have shown no statistical (P<0.05) changes. Acidifiers act as performance promoters by lowering the pH of gut (mainly upper intestinal tract), reducing potential proliferation of un-favorable microorganisms. Acidification of gut stimulates enzyme activity and optimizes digestion and the absorption of nutrients and minerals. Un-dissociated form of organic acids penetrates the lipid membrane of bacterial cells and dissociate into an ions and protons. It can be concluded that dietary acidifier agent increased the performance of broiler chicken and is an option for maintaining or improving broiler growth and efficiency. The best level of acidifier used in this study was found to be 0.1%.

Keywords: Acidifiers, MOS, Body Weight, Feed Consumption, FCR, Broilers.

INTRODUCTION

Natural immune system in newly hatched poultry is incompetent [1-3] and pathogenic bacteria such as *Enterobacteriaceae* and *Enterococcican* predominate in their gastrointestinal tract and through damaging effects on cell wall of intestine, decrease performance at the whole period of poultry life [4-6]. Scientific literature indicates an increase of over 85% in the number of infections caused by *Salmonella Enteritidis* during the last few years from products of poultry origin [7]. Because feed additives can affect microbial population in the gastrointestinal tract [8&9], they are of great interest in the poultry industry. So Antibiotics were used worldwide in poultry industry in the past 60 years for preventing diseases and improvement of growth performance. But continuous and misuses of antibiotics in livestock production and specially poultry industry resulted many concerns about development of drug-resistant bacteria, drug residues in the body of the birds, and imbalance of normal microflora. Therefore, animal researchers and animal food producers are looking for suitable feed additives to improve poultry natural immune system and hereby increase poultry performance. Several scientific reports demonstrated that acidifiers and organic acids could stimulate the natural immune response of poultry, reduce the activity of pathogenic bacteria and balance bacteria population in poultry [10-12]. It seems that the positive effects of these feed additives are mainly include: reducing colonization of pathogenic microorganisms, reducing production and releasing toxic components from bacteria and their antifungal and antibacterial activities that are totally due to an increase in broiler performance [13-15]. Acidifiers constitute an important component of modern feeding strategies without antibiotics. Organic acids are known to have strong antibacterial effects and they have been used in the protection of feed from microbial and fungal destruction. The addition of organic acids to animal feeds has been reported to decrease the intraluminal concentration of coliform bacteria and other acid-intolerant organisms, such as *Campylobacter* and *Salmonella*, known to be involved in digestive disorders [16&17]. The antimicrobial mechanism of acids is related to the reduction in pH of the environment, which limits the growth of bacteria less tolerant to acid pH. In addition, undissociated organic acids can easily penetrate the lipid membrane of bacteria and molds. In the cell, the organic acids release the protons in the alkaline cytoplasm, resulting in the decrease of intracellular pH. This alters enzymatic reactions and the nutrient transport system, forcing the bacterial cell to use energy to release protons and cause an intracellular acid anion accumulation [18]. Other benefits associated with acidification are improvements in digestive enzyme and microbial phytase activity [19], increased pancreatic secretion and stimulation of gastrointestinal cell proliferation [20]. Many studies have reported several beneficial effects showing improvements not only in the general health of animals, but also in growth rate and feed efficiency when various dietary additives were supplemented to the diet [22]. Among these alternatives, acidifiers have been considered as an attractive additive for weaning pigs' diets. It is proposed that dietary acidifiers may provide a prophylactic measure similar to feed antibiotics [22]. While antibiotics are designed to inhibit most microbial growth, acidifiers would reduce harmful microorganisms and help beneficial microorganisms to dominate in the gastrointestinal tract of commercial poultry [23]. Mannan-oligosaccharides (MOS) are mannose-rich carbohydrates found in the yeast cell wall. Currently, MOS products, particularly those derived from the cell wall of *Saccharomyces cerevisiae*, are extensively used as natural feed additives in livestock and poultry because of documented benefits in performance [24 & 25] and gastrointestinal health [26] but without the resistance-related risks associated with the use of dietary antibiotics. Several mechanisms have been proposed to explain the beneficial effects related with MOS. One popular model is based on the fact that mannose can block the colonization of intestinal pathogens, such as *Salmonella spp.* and *Escherichia coli*, which contain type 1 fimbriae with mannose-binding lectins. Mannan-oligosaccharide-bound pathogens are prevented from attaching to intestinal mannose residues. Mannan-oligosaccharide has also been linked with improved gut health, indicated by increased villi length and the number of goblet cells and increased populations of beneficial bacteria such as *lactobacilli* and *bifidobacteria* in the guts of broilers and turkeys while reducing the populations of *Salmonella* and *Escherichia coli* [23 & 26]. Another suggested mode of action of MOS is immune system modulation activity, which suggests that MOS can stimulate intestinal mucosal immunity, perhaps by acting as a nonpathogenic microbial antigen [27]. It was reported that dietary supplementation of 0.05% of MOS increased mucosal IgA secretions and humoral and cell mediated immune responses of neonatal chicks [25]. The relationship between the effects of MOS as well the dominant mode of the action remains unclear due to our limited understanding of the underlying effects of MOS at a molecular level. However, evaluation of these feed additives efficiency which contains acidifiers and MOS on immune response and performance of broiler chicks requires studies that are more comprehensive. Therefore, a feeding trial was conducted to investigate the supplementation of a Natural Growth Promoter (Acidifier Agent) on growth performance, visceral organ weight and immune response, and blood biochemical parameters of broiler chickens. The efficacy of different dosage of NGP compared with AGP was also investigated in this study.

MATERIALS AND METHODS

The present study was carried out in the Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran with an objective of assessing the performance parameters and visceral organ weights of commercial broilers fed with an acidifier agent.

Experimental design, Housing, Management and Test Diet

A total number of 240 day-old unsexed Ross 308 broiler chicks were wing banded, weighed and distributed in a completely randomized experimental design with four treatments and three replications of twenty chicks each. Each replicate group of chicks housed in an independent pen, conventional sided deep litter house. Chicks in all the replicates were reared up to six week of age under uniform standard conditions throughout the study. Brooding was done till three weeks of age using incandescent bulbs. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided *ad libitum* feed and water throughout the study. Feeding of test diets commenced at first day of age and continued till the termination of experiment at six weeks of age. The temperature was maintained at 30±1°C in the first week and reduced by 2.5°C per week to 21°C. From day one until day 4 the lighting schedule was 24 h light. At days 5-49 the dark time was increased to **1** h. Basal diet was formulated and compounded to meet the nutrient requirements of commercial broilers during the starter (0-2 wks), grower (2-4 wks) and finisher (4-6 wks) feed. The composition of experimental diets is shown in Table 1. Diets prepared without additive as Control (CON) (group1); 0.025% Acidifier Agent (AA1) (group2); 0.05% Acidifier Agent (AA2) (group 3) and 0.1% Acidifier Agent (AA3) (group4).). The natural acidifier gent used in this study was Totacid (containing citric acid, acetic acid, propionic acid, lactic acid and MOS from natural sources) provided by a commercial company (Tehran Dane Limited, Tehran, Iran).

Table 1: Ingredients and composition of the basal diets (as-fed basis, %)

Ingredients (%)	Starting diet (0-2wk)	Growing diet (2-4wk)	Finishing diet (4-6wk)
Corm	59.00	67.36	72.01
Soybean meal	33.74	28.63	24.46
Soybean oil	1.56	0.65	0.56
Calcium carbonate	0.60	0.67	0.63
Dicalcium phosphate	1.41	1.02	0.84
Oyster shell	0.66	0.66	0.63
Common salt	0.30	0.30	0.30
Vit. And Min. Permix[1]	0.50	0.50	0.50
DL-Methionine	0.13	0.06	0.02
Lysine – HCL	0.09	0.14	0.05
Calculated analysis			
ME (Kcal/kg)	2900	2950	3000
Crude protein (%)	20.84	18.43	16.87

[1]The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000 IU (all-trans-retinal); Vit. D3 (cholecalciferol), 2,000 IU; vitamin E, 20 IU (α-tocopherol); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg and antioxidant, 250 mg.

Vaccination schedule

The local office of Iranian Veterinary Organization have suggested the required local vaccination and modulated by the veterinarian of Malayer University, as below:

Vaccination against Newcastle Disease (ND) virus happened three times: first spray at the commencement of experiment, second on the 12th day as B1 (CEVA SANTE ANIMALE, Libourne, France) in drinking water and booster of them on 20th day as clone-30 (HIPRAVIAR® CLON, Amer, Spain) in drinking water. Vaccination against Bronchitis virus happened in two times as the following: first spray at commencement of the experiment and the booster in drinking water on the 10th day, both as H-120 (CEVA SANTE ANIMALE, Libourne, France). Vaccination against Infection Bursal Disease (IBD) virus happened in two times: first on day 15 and the second on the 24th day, both as Gambo-l (CEVA SANTE ANIMALE, Libourne, France) in drinking water. The sera were applied to HI test in 28 the day, to determine Ab to NDV. In titers lower that 5, the booster B1 (CEVA SANTE ANIMALE, Libourne, France) was administrated in drinking water for broilers.

Studied parameters

Performance parameters

The weekly body weight, feed consumption and mortality, if any were recorded and gain in weight and feed efficiency were calculated. All chicks were weighed individually at the end of each week till 6th week of age using digital electronic top pan balance with 1g accuracy to measure body weight. Feed consumption was recorded replicate-wise weekly in all the experimental groups up to 6 weeks of age and feed consumption per bird was estimated. Feed conversion ratio (FCR) was calculated weekly up to 6 weeks, as feed consumed per unit body weight gain. The body weight of dead birds was also included in the calculation of FCR. The number of dead birds in each replicate was recorded to measure the survivability. The dead birds were subjected to thorough postmortem examination to identify the cause of death. The per cent survivability weekly up to 6th week was computed.

Visceral organ weights

At the end of the trials, upon obtaining the permission of Ethical Committee of the University, six birds from each replicate were sacrificed by cutting the jugular vein and blood samples were individually collected in 10-mL heparinized tubes and stored on ice for hematology analysis. The visceral were then opened and the thymus, spleen, bursa of Fabreciuos, liver, kidney and pancreas removed and weighed on digital top pan electronic balance (0.1g accuracy) and the later three weighed on manopan balance (1mg accuracy). The weights were adjusted to one kg live weight and treatment means were calculated.

Statistical analysis

The experimental data were analyzed statistically by using the General Linear Model procedure of the Statistical Analysis System (SAS®) software (SAS Institute, USA, 2000). Overall data were analyzed using one way ANOVA test. Duncan multiple range test at 0.05 probability level was employed for comparison of the means (Duncan, 1955).

RESULTS AND DISCUSSION

The effects of different dosage of feed additive on body weight (g): The result s of dietary treatments on weekly body weight of broiler chicks are shown in Table 2. The initial day old weights of chicks were in a similar range, without any significant change. At 7 day, the mean of groups 3 and 4 (AA2 and AA3) was found significantly ($P<0.05$) higher, compared to control group. At the end of week II, this trend was found only in group 4 (AA3), when compared to other dietary treatments. At 21 day, groups 3 and 4 (AA2 and AA3) have shown a significant ($P<0.05$) increase in body weight, where other treatment groups have shown a non-significant when compared with control group. At day 28, the three levels of acidifier have shown a significant ($P<0.05$) change in body weights. The higher was observed in 0.1% level of acidifier agent used in this study.

Table 2: Body Weight (g) of broilers fed different levels of Acidifier Agent

Treatment groups	Week 0 (day old)	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
[1]CON	42.19^{a}	163.49^{c}	447.72^{b}	863.38^{c}	1379.24^{b}	1952.29^{c}	2590.48^{b}
[2]AA1	42.23^{a}	162.43^{c}	448.24^{b}	864.46^{c}	1382.36^{b}	1960.34^{b}	2589.61^{b}
[3]AA2	42.64^{a}	167.36^{b}	450.34^{ab}	870.43^{b}	1388.52^{ab}	2016.41^{a}	2631.38^{a}
[4]AA3	42.36^{a}	171.28^{a}	452.23^{a}	878.90^{a}	1390.35^{a}	2018.34^{a}	2635.24^{a}
SEM	0.223	0.227	0.083	0.546	0.094	0.119	0.748

Mean values within a row with different superscript letters (a, b and c) were significantly different (p<0.05). [1]CON (Control); [2]AA1 (Acidifier Agent @ 0.025%); [3]AA2 (Acidifier Agent @ 0.05%) and [4]AA3 (Acidifier Agent @ 0.1%, respectively). SEM: Standards Means of Errors.

At the end of week V, all three levels of acidifier fed groups have shown a significant ($P<0.05$) increase in body weight of broilers, when compared with control. At the end of the last week, (day 42), the last two treatment groups (AA2 and AA3) had shown a significant increase in body weight, where the 0.025% level remained non-significant, when compared with control group.

Table 3: Feed intake (g/bird) of broilers fed different levels of Acidifier Agent

Treatment groups	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
[1]CON	155.96^{a}	554.72^{a}	1244.27^{a}	2175.06^{a}	3385.27^{a}	4893.41^{a}
[2]AA1	154.63^{a}	506.06^{b}	1182.58^{b}	2018.24^{b}	3214.95^{d}	4562.89^{c}
[3]AA2	150.79^{a}	499.42^{b}	1171.59^{b}	2002.24^{c}	3284.73^{b}	4633.86^{b}
[4]AA3	154.49^{a}	497.90^{b}	1181.24^{b}	2002.10^{c}	3263.65^{c}	4609.03^{b}
SEM	0.436	0.528	0.172	0.204	0.098	0.327

Mean values within a row with different superscript letters (a, b and c) were significantly different (p<0.05). [1]CON (Control); [2]AA1 (Acidifier Agent @ 0.025%); [3]AA2 (Acidifier Agent @ 0.05%) and [4]AA3 (Acidifier Agent @ 0.1%, respectively). SEM: Standards Means of Errors.

The effects of feed additive on feed in take (g/bird): The results of dietary treatments on weekly feed intake of broiler chicks are shown in Table 3. It is noteworthy that the results indicated that at the end of first week, FI remained non-significant among all treatment groups, when compared with control group. The scenario was totally changed for day 14 and day 21, where all 3 treatments fed different levels of acidifier had a significantly ($P<0.05$) decreased FI, when compared with their respected control groups. On day 28, the FI decreased in all 3 levels of acidifier and have shown a significant ($P<0.05$) decrease, with more emphasis on AA3 level. At the end of week V, the three levels of acidifier fed groups have shown decrease in FI, statistically ($P<0.05$), with an emphasis on the AA1group.

The same trend was found at day 42, where the minimal FI was found in AA1 group, where all three acidifier fed groups have shown a significant (P<0.05) reduction in FI.

The effects of feed additive on FCR: The results of dietary treatments on weekly FCR of broiler chicks are shown in Table 4. All of the birds fed feed additive had numerically better FCR than the control birds. In particular, at the end of week I, AA2 and AA3 treatment groups have shown a significant (P<0.05) decrease in FCR of broilers.

Table 4: Feed conversion ratio (FCR) of broilers fed different levels of Acidifier Agent

Treatment groups	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
[1]CON	0.954^{a}	1.239^{a}	1.418^{a}	1.577^{a}	1.734^{a}	1.889^{a}
[2]AA1	0.952^{a}	1.129^{b}	1.368^{b}	1.460^{b}	1.640^{b}	1.762^{b}
[3]AA2	0.901^{b}	1.109^{c}	1.346^{c}	1.442^{c}	1.629^{c}	1.761^{b}
[4]AA3	0.902^{b}	1.101^{c}	1.344^{c}	1.440^{c}	1.617^{d}	1.749^{c}
SEM	0.344	0.577	0.923	0.036	0.034	0.923

Mean values within a row with different superscript letters (a, b and c) were significantly different (p<0.05). [1]CON (Control); [2]AA1 (Acidifier Agent @ 0.025%); [3]AA2 (Acidifier Agent @ 0.05%) and [4]AA3 (Acidifier Agent @ 0.1%, respectively). SEM: Standards Means of Errors.

For the rest of the trial, FCR of broilers of which have fed different levels of acidifier have shown a significant (P<0.05) decrease in FCR, when compared with their respected control groups. However, among the acidifier fed treatments, numerical and sometimes significant changes were present.

The effects of feed additive on survivability (%): The results of dietary treatments on weekly survivability percentage of broiler chicks are shown in Table 5. At the end of first week, the survivability of broiler chicks fed different levels of acidifier had shown a 100%, except in AA3, where the percentage of 99 is recorded. At the end of week II, the survivability percentage for control, AA1 and AA2 were observed 99 and in AA3 group was observed 98. At day 21, no changes in control and AA1treatment groups were observed, however, in AA2 and AA3 groups, the 98 and 97 percent were seen, respectively. At day 28, the recorded percentage mortality in different groups were, CON and AA1: 98, AA2: 97 and AA3: 96. At the end of fifth and sixth week, for the control, AA1, AA2 and AA3, the percentage of 96, 98, 96 and 95 were recorded, respectively.

Table 5: Survivability rates (%) of broilers fed different levels of Acidifier Agent

Treatment groups	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
[1]CON	100	99	99	98	96	96
[2]AA1	100	99	99	98	98	98
[3]AA2	100	99	98	97	96	96
[4]AA3	99	98	97	96	95	95

Mean values within a row with different superscript letters (a, b and c) were significantly different (p<0.05). [1]CON (Control); [2]AA1 (Acidifier Agent @ 0.025%); [3]AA2 (Acidifier Agent @ 0.05%) and [4]AA3 (Acidifier Agent @ 0.1%, respectively). SEM: Standards Means of Errors.

Table 6: Visceral Organ weights (g/kg live weight) of broilers fed different levels of Acidifier Agent at 42 days

Treatment groups	Thymus	Spleen	bursa of Fabreciuos	Liver	Kidney	Pancreas
[1]CON	4.18^{a}	1.43^{a}	1.49^{a}	27.98^{a}	7.48^{a}	4.51^{a}
[2]TAA1	4.24^{a}	1.42^{a}	1.46^{a}	28.03^{a}	7.45^{a}	4.53^{a}
[3]TAA2	4.23^{a}	1.39^{a}	1.47^{a}	28.06^{a}	7.47^{a}	4.49^{a}
[4]TAA3	4.14^{a}	1.41^{a}	1.48^{a}	28.05^{a}	7.42^{a}	4.52^{a}
SEM	0.253	0.037	0.034	0.165	0.226	0.098

Mean values within a row with different superscript letters (a, b and c) were significantly different (p<0.05). [1]CON (Control); [2]AA1 (Acidifier Agent @ 0.025%); [3]AA2 (Acidifier Agent @ 0.05%) and [4]AA3 (Acidifier Agent @ 0.1%, respectively). SEM: Standards Means of Errors.

The effects of feed additive on visceral organ weights (g/kg live weight): The results of dietary treatments on visceral organ weights of broiler chicks at 42 days of age are shown in Table 6. The thymus weight varied from 4.14 to 4.24 and no significant changes were observed between the dietary treatments. The spleen weights were varied from 1.39 to 1.43 and no significant changes between the treatments were noticed. No significant differences were recorded for bursa of Fabreciuos weight and the weights were recorded between 1.46 and 1.49.

The liver weights-in a similar trend- were varied from 27.98 to 28.06 and kidney weights recorded from 7.42 to 7.48, where no statistical differences were noticed among all treatment groups. The pancreas weights were varied from 4.49 to 4.53, without showing any significant changes among the dietary treatment groups. The results of the present study are in agreement with the findings of other scientists across the globe. Similar effects were found by other researchers, who reported that organic acids and probiotics have no significant effect on BWG of broiler chicks [22 & 30]. However, other researchers reported beneficial effects of these additives on BWG [26]. The same trend was also reported by [27], who reported that the acidifier affected the body weight of the chicken from week I until the termination of the experiment (week 5). Another study reported that although, there were no differences in body weight (BW), but body weight gain and feed intake among treatments from day1to 21, FCR in birds fed acidifier was lower than the control group (P<0.0001) [21]. Because pattern of FI in birds is based on energy level intake, it is likely that the birds which have better FCR have a lower FI. Therefore, this effect may be related to this assumption. In accordance with this experiment, observations of other researchers indicated that the addition of probiotics and organic acids to the broilers diet either numerically or significantly improves FI [11]. The effect of FCR may be due to higher BWG and lower FI in the birds fed acidifier. In addition, it is reported that decreasing of pH of digestive organs using organic acids and probiotics could lead to better digestion, absorption and utilization of nutrients [21 & 30 & 18]. Acidifiers act as performance promoters by lowering the pH of gut (mainly upper intestinal tract), reducing potential proliferation of un-favorable microorganisms. Acidification of gut stimulates enzyme activity and optimizes digestion and the absorption of nutrients and minerals. Un-dissociatccl forms of organic acids penetrate the lipid membrane of bacterial cells and dissociate into anions and protons. After entering the neutral pH or the cells cytoplasm, organic acids inhibit bacterial growth by interrupting oxidative phosphorylation and inhibiting adenosine tri-phosphate in organic phosphate interactions. In another study [24], the body weight of broilers supplemented with acidifier was significantly (P<0.05) higher than broilers in control group on day 28. At the end of the experiment (day 42), broilers supplemented with acidifier had higher body weight in compare to control group (P<0.05). Martins et al. (2005) found that citric acid supplementation as an acidifier caused a significant increase on body weight in broiler chickens [15]. Kishi et al. (1999) found that the addition of dietary citric acid, acetic acid, or lactic acid improved body weight of broiler chickens compared with control group [13]. The inclusion of acidifier in broilers has often showed growth performance increases [18 & 31] and dosage rates for commercially produced acidifiers are recommended between 0.2% and 1.0%. The mode of action of acidifier in poultry is mainly due to its antimicrobial action, unlike in pigs where a key activity is the reduction of the stomach - pH. The effect of acids on gram-negative bacteria is increased if the organic acid is not dissociated. Because of this mode of action, effective acidifiers need to contain organic acids that are undissociated at different pH-values, so that the anti-microbial action is prolonged over a wider pH range. In the trials discussed, final body weight of the broiler chickens fed acidifier diets was significantly increased. Average feed consumption was higher in the acidifier group, and FCR was slightly reduced, even though this reduction was not significant.

CONCLUSION

It can be concluded that the addition of a balanced acid blend, such as combinations of lactic acid, formic and propionic acid based on a sequential release medium, increases the performance of broiler chicken and is an option for maintaining or improving broiler growth and efficiency results without resorting to supplementation with an AGP. Based on the results of this experiment, acidifier has the potential to be used as feed additives in broiler diets. The results also revealed that the best level of acidifier used in this study was found to be 0.1%. However, further studies are needed to amplify the results of this experiment and to determine whether these results are likely to be applicable for other rearing conditions.

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