by Sri Kismiati

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#### Effectiveness of Acidifier in Broiler Fed Diet Double Step-Down Protein

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Luthfi Djauhari Mahfudz<sup>1</sup>, Teysar Adi Sarjana, Sri Kismiati, Rina Muryani, Maulana Hamonangan Nasoetion and Nyoman Suthama

<sup>1</sup>Faculty of Animal and Agriculture Sciences, Diponegoro University

E-mail: inditik@yahoo.com

Abstract. Research' aim was to determine the effectiveness of acidifiers in broiler fed double stepdown protein. A total of 168 birds (84 males and 84 females, initial BW was  $186.3 \pm 0.68$  g) were raised for eight weeks. The first 7 days, chickens were fed on commercial feed. Experimental diets were composed to contain protein and energy of 21.41% and 2.856.91 kcal/kg, for starter control diet, and of 19.15% and 2884.12 kcal/kg for starter dietary protein step-down similar to finisher control diet, and of 17.37% and 2882.13 kcal/kg for finisher protein step-down diet, respectively. The study was arranged in a completely randomized design with seven treatments and four replications (6 birds each). Parameters measured were feed and protein consumptions, protein digestibility, body weight gain, feed conversion, and income over feed cost. Data were analysed by variance and to Duncan multiple range test. The results showed that dietary inclusion of acidifiers lowered feed and protein consumptions, but it increased body weight and income over feed cost as well as improved feed conversion. In conclusion, acidifiers inclusion, especially at the level of 0.8% synthetic citric acid, in the double step-down protein can increase efficiently broiler performance and improves income over feed cost.

#### 1. Introduction

To support the fast growth rate of broiler chickens, the provision of nutrients, especially protein, is important. However, the dietary protein content is positively related to the price of feed, which implies that the higher dietary protein content, the more expensive price of the feed. It is known that feed cost for broiler chickens contributes up to 70% of the total production costs. In order to reduce the cost of production, it is, therefore, necessary to manipulate the feed, for instance by reducing the level of protein content (step-down). Lowering dietary protein content at starter period decreased feed consumption which brought about the improvement of feed conversion ratio (FCR) compared to controls [1]. Decreased levels of protein feed lower than the standard can be done via two ways. The first, a decrease in protein feed at the starter alone known as single-phase step-down protein, and the second, the decrease in feed protein in both starter and finisher periods called as double step-down protein. However, in most cases, decreased protein level is often accompanied by the impairment of broiler performances. In this case, decreasing dietary protein content should be followed by the improvement of the absorption of protein in order to fulfil the protein needs of broiler for growth. Previuos studies demonstrated that the improvement

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of protein absorption can be achieved by dietary inclusion of acidifier when the ration of decreased protein was fed [2] [3].

Acidifieris an organic acid which is beneficial in preserving and protecting the feed from destruction of microbes and/or fungi. It also has a direct impact on the improvement of feed or nutrients digestibility in chickens. The mechanism of action by which the acidifier improves the digestibility of nutrients is not clearly known, but it may decreases the gastric pH and reduces the microbial pathogens in the gastro intestinal, particularly gram-negative bacteria. That in order to improve the performance of broiler, the ideal addition of formic acidas acidifier should be less than 0.5% [4]. This slightly differs from other reference suggesting that the ideal dose of commercial acidifier ranges from 0.2% to 1% of feed. Previous study showed that there was a same effect between organic acidsof formic and propionic acidsat the level of 0.3% on growth of broiler at 35 days of age [5]. Thus, acidifier can be assumed to serve as a substitute for antibiotics as growth promoters for broiler chickens.

The indicators of broiler performance can be based on feed intake, body weight gain, and feed conversion, while those of effectiveness can be evaluated through protein digestibility and income over feed cost (IOFC). The body weight of broiler increased and followed by the improvement of feed conversion with the increased level of dietary citric acid [4]. Moreover, the level of 0.8% total acid lemon extract provided the best impact on the microflora and intestinal characteristics as well as performance of broiler production [6]. Based on the above phenomenon, the present study was conducted to examine the effectiveness of inclusion of both synthetic and natural citric acid derived from lemon juice as acidifier in the step-down protein diet on the performance and income over feed cost of broilers.

#### 2. Materials and Methods

Experimental Animal, Feed and Design.

A total of 168 birds (84 males and 84 females); initial body weight was  $186.3 \pm 0.68$  g of 8 days old broiler chickens of Lohman MB 202 strain were raised for eight weeks. From day 1 to 7, chickens were provided commercial crumble-form feed containing 21-23% of crude protein (CP) and 2900 kcal/kg of metabolizable energy (ME). From day 8 thereafter, chickens were given experimental feed until the completion of the study. Feed composition and nutrients content are presented in Table 1.

The study was arranged in a completely randomized design (CRD) with seven treatments and four replications. Each replication consisted of three males and three females and the birds were kept at a cage of 1 x 1 x 0.6 m in size. The basal diet implemented in the present study was double step-down of protein for both starter and finisher periods.

The overall treatments were P0: control feed (without step-down and citric acid); P1: double step-down feed without citric acid; P2: double step-down feed+0.8% lemon citric acid; P3: double step-down feed+0.4% synthetic citric acid; P4: double step-down feed+0.8% synthetic citric acid; P5: double step-down feed+1.2% synthetic citric acid; P6: double step-down feed+1.6% synthetic citric acid.

Parameters measured were feed consumption from 2-6 weeks (amount of feed consumed substracted by residual feed), protein consumption (feed consumption multiplied by feed protein content), protein digestibility (protein consumed substracted by fecal protein and devided by amount of protein consumed times 100%), body weight gain, average feed conversion, and income over feedcost (income derived from selling items substracted by feed costs). Data were subjected to analysis of variance (ANOVA) and when the treatment indicated significant effect, it was continued to Duncan's multiple range test (P <0.05).

#### 3. Result and Discussion

In general, the average feed consumption of broiler found in the present study 3674.81g/bird. The average feed consumption during the study was also much lower as compared to the standard of Lohmann broiler strains [7], which could reached to be 3670 g/bird on week 5. The low feed intake of broilers could be due

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to the high ambient temperatures (34°C) during the feedingtrial. High ambient temperature negatively affected feed intake in order to reduce the heat load of the body [8]. Body weight gain in average observed in the present study was low, in which body weight gain of 42 days old broilers was 2045.25 g/bird [9]. It is clear that the different results of the present study seemed to be due to the relatively low feed intake (Table 2) and high ambient temperature as it has been discussed previously.

When compared among treatments, the results of this study demonstrated that the double step-down treatment with the provision of acidifier had significantly affect feed intake of broiler. P2 and P5 were significantly lower (P < 0.05) than P0. Chickens provided dietary treatments of P0 and P1 had the highest total feed intake than chickens in P2 treatment. This condition could be due to the decrease in feed protein causing an increase in feed intake of broilers. The phenomenon obtained in present study was disagreed with [10] who reported that the decreased amount of protein in the diet improved feed consumption in laying hens. Dietary inclusion of citric acid as an acidifier tended to decrease feed intake and provided an impact on the significantly (P < 0.05). It decreases in protein intake (Table 2). This result was consistent with the report of Abdel-Fattah et al (2008) and Kopecky (2012) that the administration of citric acid in the diet caused the decreased feed consumption inbroilers [11] and [12]. However, the effect of citric acid on feed consumption is presumably dose-dependent as reported by Haque et al (2009) that organic acids at high concentrations (1.2%) could reduce the palatability of the feed, and, therefore, consumption also reduced [12].

The color of feed can be an important determinant for feed consumption due to the inclusion of acidifier in poultry. The higher acidifier concentration included in the feed, caused the darker colour of the feed, as the citric acid was administrated in the liquid form. Although birds prefer to the brighter than the darker feed [13] and thus may lower the feed consumption, addition of citric acid has been assumed to beable to increase the activity of digestive enzyme. The efficiency of protein utilizations due to acidifier inclusion (P2 to P6) were better than that of non-acidifier treatment (P0). Treatment of dietary protein step-down without acidifier (P1) resulted in the highest feed consumption (Table 2), but body weight gain was equal to P3 to P6 (step-down protein plus acidifier) although withlower feed consumptionand protein intake (P <0.05). This suggests that feeding acidifier can improve nutrient efficiency, especially protein. On the other hand, protein intake of the P1 treatment was significantly highest value among others due to the high feedconsumption, but body weight gain was lower. This phenomenone suggests that nutriens, especially protein, of the treatment without acidifier was not utilized as efficient as that with citric acid.

Feeding double step-down protein diet with the addition of acidifier significantly (P <0.05) improved protein digestibility (Table 2). Although dietary protein was reduced both at the starter and finisher periods (double step-down protein), acidifiers was able to increase protein absorption as indicated by higher body weight gain. The increase in nutrient (protein) utilization efficiency can be connected with the improvement of gastrointestinal ecology and morphology. Natural citric acid derived from lemon juice was reported to be able to suppress the growth of pathogenic bacteria and toimprove intestinal morphology, especially villi of the duodenum [2], and finally improving the digestibility and absorption of nutrients. The improved microbes balance in the intestinal environment can be connected to lowered duodenal pH from 6-7 to 4-5 caused by the addition of 0.8% citric acid. The improvement of small intestine ecology due to properly natural citric acid supplementation was indicated by the reduced *Eschericia coli* population, and on the contrary, the number of lactic acid bacteria (a non-phatogenic) increased. Zang et al (2009) stated that the growth of small intestinal villi are closely related to body weight gain of broilers, as the higher villi growth the greater chance of nutrient(s) absorption [14].

Organic acid in general is very potential to reduce harmful (pathogen) bacteria and changes the intestinal morphology which in turn increases the supply of nutrients to the host [15]. It has been described in the previous paragraph that dietary inclusion of citric acid significantly (P<0.05) decreased protein intake as an impact of slightly reduced feed consumption. However, on the other hand, body weight gain was not impaired by the feeding diet with acidifier inclusion, even numerically it tended to

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improve. The lower protein intake (due to the low feed consumption) may be compensated by the improved absorption of protein after administration of acidifier [16]. This suggests that the addition of organic acids in broiler feed could improve nutrient utilization, growth and feed efficiency [12]; [3]; [17]. Protein consumption (Table 2) is slosely related to the metabolism of protein, especially protein deposition processes, for growth acceleration. The amount and quality of feed protein are the important factor affecting the efficiency of protein utilization for production or growth [18]; [19]. The condition in the present study was consistent to previous finding [20] that the availability of protein had an important role in the process of accelerating the rate of protein synthesis supporting growth of birds. Decreasing feed consumption followed by protein intake didn't reduce growth or body weight gain as compared to control. It was different from the report of Ma'rifah et al (2013) that the decrease in protein intake followed by N retention reduced meat protein mass and body weight gain of broiler chicken fed Salvinia molesta without supplemental acidifier [21]. However, reduced protein intake did not interfere with the meat protein mass atributable to the growth performance when feed was added with citric acid of lime juice [2].

Organic acid is also known to have antibacterial properties, and the basic principle mechanism of antibacterial action is that penetrate the bacterial cell wall and disrupt the normal physiology of certain types of bacteria, especially low pH sensitive bacteria. Therefore, organic acids can be used as growth promoter via its function in stabilizing the microflora in the digestive tract, and improve performance of birds in general [22]. The efficient growth improvement depended on the deposition of dietary protein into the meat which is very beneficial effect in broiler industry [19]; [23]. The increased protein digestibility as a substrate for body protein synthesis, it is a primary consideration in accelerating growth of poultry in the tropics [20]. Growth acceeleration was impossed by the difference between rates of protein synthesis and degradation [24], under the contributing amount of protein supply derived from the digestion and absorption. The availability of protein as a substrate is closely related to the metabolism of protein together with calcium known as calcium-binding protein (Ca-BP). Function of acidifier property by lowering the pH and by improving the health status of digestive tract would be beneficial for the increasing nutrients absorption, especially protein and Ca. Considering pH of digestive tract can be lowered by acidifier inclusion, therefore, the administration of citric acid can increase the absorption and transport of mineral calcium [11] [25]. The anions of the acids can bind to calcium resulting in acidic conditions; thus calcium can be absorbed by the intestine easily [26]. The increase in Ca absorption is suported by the improved protein intake and digestibility due to feeding acidifier (Table 2), both nutrients are essential for the growth of broiler, and this bionutritional mechanism was proved by the body weight gain tended to be higher.

Reduction dietary protein without additional acidifier (P1) produced the significantly (P<0.05) highest feed conversion ratio (FCR) due to the more feed was consumed with relatively low body weight gain (Table 2). High FCR valuesupported by the high protein intake in P1broilers group was the evidence of the inefficiency of feed or nutrient utilization. It can be concluded that nutrient, especially protein, utilization was less effective when the birds fed reduced dietary protein without addition of citric acid, as compared to citric acid-treatedgroups (P3 to P6). Slightly different phenomenon was found in broilers given standard diet as a control (P0), showed that body weight gain and FCR were similar to those of ciric acid-treated birds since feed consumption was the same but protein intake was the highest. Feeding standard diet which was formulated to meet protein requirement of the chickens brough about the higher productive cost because the higher protein content of the diet, the more expensive cost of feed. On the other hand, additional citric acid derived from lemon juice in dietary protein step-down at the level of either 0.8% (P4) or 1.2% (P5) were able to produce body weight gain similar to that of control but with improved FCR. As it has been discussed previously that the increased efficiency of feed or nutrient utilization due to dietary inclusion of citric acid giving the impact of growth improvement. Providing dietary acidifier was reported to increase the surface area of the intestinal villiin facilitating the more

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nutrients that can be absorbed [27], and this process resulted higher body weight and lower FCR. Similarly, the organic acid supplementation could improve nutrient utilization resulting in higher body weight gain and finally lowered feed conversion [4]. Therefore, feeding management with double stepdown dietary protein combined with additional citric acid as acidifier improved efficiency of feed utilization and growth, as characterized by low FCR. Feed conversion ratio is the ratio of the amount of feed consumed and body weight gain, thus the smaller the FCR value, the more efficient utilization of the feed [28].

Feeding management by providing low protein diet either in the starter or finisher periods, known as double step-down protein diet, added with acidifier significantly (P < 0.05) increased revenue, as indicated by the income over feed cost (IOFC). The IOFC values of P3 to P6 treatments were significantly (P <0.05) higher compared to those of P0 and P1 groups. Feed consumption and protein intake were lower with the same protein digestibility (Table 2) in the citric acid-treated groups, but it resulted in relatively better body weight gain and higher IOFC. Prahadi et al (2015) showed that additional of Averrhoa bilimbi juice in the diet of laying hens, significantly increasing feed intake, Hen Day Production, and Income Over Feed Cost of laying hens [29]. The combination of feeding step-down protein dietin both starter and finisher periods with additional citric acid can lowered feed costs and increased IOFC. Therefore, this results of study provided an understanding that the combination of step-down protein diet and citric acid plays an important role for both productive improvement and economically profitable.

#### 4. CONCLUSION

It can be concluded that inclusion of citric acid at the level of 0.8% (P4) resulted a better productive performance and higher profits in broiler chickens fed double step-down protein diet.

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Table 1. Composition and nutrients content of the experimental feed

		Experim	ental feed	
T 17 1 1	St	arter	Finisher	
Feed Ingredients	Normal	Step Down	Normal	Step Down
Yellow Corn	48.00	53.00	53.00	53.50
Rice Bran	14.00	16.00	16.00	21.50
Vegetable Oil	2.00	1.00	1.00	0.50
Soybean Meal	28.00	22.00	22.00	16.50
Fish Meal	6.50	6.50	6.50	6.50
CaCO <sub>3</sub>	0.50	0.50	0.50	0.50
Shellfish Flour	1.00	1.00	1.00	1.00
TOTAL	100.00	100.00	100.00	100.00
Nutrient Content (%)*				
Metabolic Energy (kcal/kg)** Crude Protein	2,856.91 21.41	2,884.12 19.25	2,884.12 19.25	2,882.13 17.37
Crude Fiber	5.09	5.18	5.18	5.75
Ether Extract	6.04	6.33	6.33	6.37
Lysine***	1.41	1.23	1.23	1.09
Metionine***	0.43	0.40	0.40	0.37
Arginine***	1.53	1.34	1.34	1.17
Ca total	1.00	0.98	0.98	0.95
P total	0.41	0.43	0.43	0.45

Composition and nutrients content of step-down starter diet was similar tothose of normal Description: finisher diet

<sup>\*</sup>Analyzed result of the Laboratory of Animal Nutrition and Feed Science, Faculty of Animal and Agriculture Sciences, Diponegoro University (2013)

<sup>\*\*</sup>The ME (kcal/kg) =  $40.81 [0.87 (2.25 \times OD + CF + EM + k] [31]$ 

<sup>\*\*\*</sup>Based on Table of Nutrient Contents of Feed Ingredients [32]

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Table 2. Feed and Protein Consumptions, Body Weight Gain, Feed Conversion Ratio, Protein Digestibility, and Income Over Feed Cost (IOFC) of Broiler Chickens as effected by treatment.

Treatment	Feed	Protein	Body	Feed	Protein	IOFC
S	Consumption	Consumption	Weight Gain	Conversion	Digestibility	(Rp)
	(g/bird)	(g/bird)	(g/bird)	Ratio	(%)	
P0	$1,960.41 \pm 55.83^{a}$	390,90±10.28a	1,059.96±27.99ab	$1.85\pm0.01^{\circ}$	$68.77^{b}$	8,709.46°
P1	$1,998.44 \ \pm 88.37^a$	$358.39{\pm}16.06^b$	$938.43 {\pm}\ 50.80^{c}$	$2.13{\pm}0.13^a$	64.86 <sup>b</sup>	7,980.38°
P2	1,789.01±11.92°	297.49±42.43°	946.47±52.34bc	$1.75 \pm 0.05^{\circ}$	$68.50^{b}$	10,425.02 <sup>b</sup>
P3	$1{,}905.69{\pm}56.50^{ab}$	342.14±10.04°	$972.04 {\pm}~48.70^{bc}$	$1.96 {\pm}~0.08^b$	$71.58^{ab}$	11,363.65 <sup>a</sup>
P4	$1{,}885.09{\pm}71.21^{ab}$	337.84±13.02°	$1,\!016.42{\pm}71.07^{abc}$	$1.86 {\pm}~0.06^{c}$	$74.30^{a}$	11,334.08 <sup>a</sup>
P5	1,832.38±72.90 <sup>bc</sup>	329.58±13.32°	$1,\!048.97{\pm}53.95^{ab}$	$1.75 \pm 0.04^{\circ}$	74.65 <sup>a</sup>	11,423.60a
P6	1,889.77±59.25ab	339.85±12.03°	1,074.36± 53.99 <sup>a</sup>	1.76± 0.04°	73.88 <sup>ab</sup>	10,390.05 <sup>b</sup>

a-cMean values in the same collumn with different superscript indicate significantly different (P<0.05)

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