

THE EFFECT OF PROPIONIC ACID ADDITION IN POULTRY DIETS ON THE INTERNAL ORGANS OF BROILER CHICKENS

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ABSTRACT

This study aims to determine the effects of adding propionic acid in the poultry diets on the internal organs of broiler chickens. The livestock used were 180 broilers with Cobb strain. This study used a completely randomized design (CRD) consisting of 3 treatments and 6 replications. The treatments in this study consisted of: P0= commercial feed without propionic acid addition; P1= commercial feed with addition of 0.5% propionic acid; and P2= commercial feed with 0.75% propionic acid. The parameters observed included live weight, spleen percentage, gizzard percentage, liver percentage, and small intestine length. The results showed that the addition of propionic acid in the broiler feed had a significant effect ($P < 0.05$) on the live weight and had no significant effect ($P > 0.05$) on the percentage of spleen, percentage of gizzard, percentage of liver, and small intestine length. The conclusion of this study was that the addition of propionic acid in poultry feed at a dose of 0.75% did not negatively affect the internal organs and the length of the small intestine of broiler chickens.

Key words: broiler chickens, physiological organs, propionic acid

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh penambahan asam propionat dalam ransum terhadap organ dalam ayam broiler. Ternak yang digunakan adalah ayam broiler strain Cobb berjumlah 180 ekor. Dalam penelitian ini digunakan rancangan acak lengkap (RAL) yang terdiri atas 3 perlakuan dan 6 ulangan. Perlakuan pada penelitian ini terdiri atas; P0= ransum komersial tanpa penambahan asam propionat; P1= Penambahan 0,5% asam propionat dalam ransum komersial; P2= Penambahan 0,75% asam propionat dalam ransum komersial. Parameter yang diamati meliputi bobot hidup, persentase limpa, persentase gizzard, persentase hati, dan panjang usus halus. Hasil penelitian menunjukkan bahwa penambahan asam propionat dalam ransum ayam broiler berpengaruh nyata ($P < 0,05$) terhadap bobot hidup dan tidak berpengaruh nyata ($P > 0,05$) terhadap persentase limpa, persentase gizzard, persentase hati dan panjang usus halus. Kesimpulan penelitian bahwa penambahan asam propionat dalam ransum dengan dosis 0,75% tidak berpengaruh negatif terhadap organ dalam dan panjang usus halus ayam broiler.

Kata kunci: ayam broiler, organ fisiologis, asam propionat

INTRODUCTION

To produce a good broiler chicken production, efforts are needed to maintain the health of the digestive tract of the animal. Generally, farmers add Growth Promoter Antibiotic (GPA) as a feed additive. The GPA can improve digestibility in broiler chickens by maximizing absorption of nutrients in the digestive tract, reducing production of toxins from digestive tract bacteria, and reducing the occurrence of infections in the digestive tract (Julendra *et al.*, 2010). According to Daud (2005), the use of antibiotics as additives in animal feed can leave antibiotic residues that might be toxic to consumers. These residues can create resistant microorganisms in humans and livestock, especially pathogenic bacteria such as *Salmonella*, *Escherichia coli*, and *Clostridium perfringens*. When these bacteria infect human or livestock bodies, the resulting diseases will be difficult to cure. Therefore, the use of a safe feed additive is very important so that there is no bad impact on consumers. The type of feed additive that is safe to use is organic acids as they leave no residue. When added to the feed, organic acids will improve the performance of the digestive organs because they increase the quality of digestive enzymes, lowering the gastrointestinal pH, and reduce the number of pathogenic bacteria in the digestive tract (Roth and Kirchgessener, 2003).

One type of organic acid that can be added to animal feed is propionic acid. This acid can improve and

maintain the condition of the digestive tract of monogastric livestock. Lueck (1980) as cited in Papatsiros and Bilinis (2014) reported that organic acids commonly used in animal feed are formic, acetic, propionic, and lactic acids, all of which have specific abilities to penetrate bacterial cell walls and kill bacteria. According to Lu *et al.* (2003) propionic acid has the ability to inhibit fungi, increase the growth of good bacteria in the digestive tract, reduce the pH of the digestive tract so as to stimulate digestive enzyme activity, and maintain a healthy condition, and microflora balance in the digestive tract. The microflora in the digestive tract plays an important role in the productivity and health of livestock, absorption of nutrients, pathogenicity, and immunity. According to Hardy (2003), propionic acid can carry out the ionization process easily by releasing hydrogen. The increase in the number of hydrogen ions will reduce the pH of the digestive tract of monogastric livestock, so that microorganisms that cannot tolerate acidic conditions will experience slow growth or die. Khosravi *et al.* (2012) reported that propionic acid can be used as a substitute for GPAs in broiler feed during maintenance.

The ability of propionic acid to maintain the condition of the digestive tract might affect the activity of internal organs that play an important role in the process of absorption of feed substances. Rimbawanto *et al.* (2019) reported that the addition of natural acidifier-based organic acids in the form of citric acid and lactic acid in poultry feed showed the same effect on the

weight of small intestine and internal organs of broiler chickens. This is because the absorption process of food substances runs normally due to unaffected weight of chicken internal organs. There are still few studies that discuss the use of propionic acid as a feed additive in poultry and its effects on physiological organs and the digestive tract. A good digestive tract condition indicates a good condition of the body and digestive organs.

MATERIALS AND METHODS

This study used 180 day-old broiler chickens (DOCs) strain Cobb produced by the PT. Satwa Unggas Jaya. The DOCs were randomly assigned into 18 experimental cage plots consisting of 10 animals each. The equipments used were feeding and drinking containers, postal cages sized 120 cm x 150 cm x 65 cm, artificial heater (brooding), digital scale, cutting knife, ruler or measuring tape and chicken baskets. The feeds used were HI-Pro (PT. Charoen Pokphan Indonesia Tbk) given to the chicken in the starter-grower phase (aged 1-21 days), MRI-P (PT. Cj Cheiljedang Feed Lampung) given to the chicken at the finisher phase (aged 22 to 28 days), and propionic acid (Zetox) produced by PT. Sehat Cerah Indonesia. The feeds were given according to the treatment whereas the water was given *ad libitum*. The nutrient composition of the feeds used is presented in the Table 1.

Research Methods

This study used a completely randomized design (CRD) consisting of 3 treatments and 6 replications. The treatment in this study was the addition of propionic acid in the feed, namely: P0= commercial feed without the addition of propionic acid, P1= commercial feed + propionic acid 0.5%, P2= commercial feed + propionic acid 0.75%. Before the chickens were brought in, the cages were first cleaned from pests and germs. The cage equipments were cleaned with water that had been added with disinfectant. The litter mat was prepared by using the husks. The feed and drinking containers were prepared.

The cage was closed with a curtain and the heater was turned on until a hot temperature ranged from 28-30° C was evenly distributed in the cage.

After 4 weeks all chickens were slaughtered. Before the slaughter, the chickens were left without food for 6 hours. The chickens were weighed to determine their live weight and then plucked. Feathers cleaning were done after dipping the chicken in warm water (30-50° C) for 45 seconds (until wing feathers were easily removed). After cutting the base of the neck and the knee joints, the abdominal cavity was opened, and the entire digestive organs was collected by holding the proventriculus and pulling out the entire digestive tracts. The internal organs (liver, gizzard, and spleen) and digestive tract were separated and cleared from fatty tissue. The length of the intestine was measured and the percentage of weight internal organs to the live weight was calculated.

Observed Variables

The live weight, spleen percentage, gizzard percentage, liver percentage, and small intestine length were measured using the following formula: Live weight (g), obtained from weighing the chickens before slaughter and after 6-hour fasting; Spleen percentage, obtained by dividing spleen weight (g) with live weight (g) multiplied by 100%; Gizzard percentage, obtained by dividing gizzard weight (g) with live weight (g) multiplied by 100%; Liver percentage, obtained by dividing liver weight (g) with live weight (g) multiplied by 100%; and Small intestine length (cm), including the duodenum, jejunum and ileum was determined by using a measuring tape. The length of duodenum was measured from the base of the gizzard to the bile duct junction. The length of jejunum was measured from the bile duct junction to the Meckel's diverticulum. The length of ileum is measured from the Meckel's diverticulum to the cecum branching (Hamsah, 2013).

Data Analysis

The data obtained were statistically analyzed using analysis of variance. If the treatment showed a

Table 1. Nutrient composition of broiler chicken feed during the study

Feed substances	Feed HI-PRO*	Feed MR1-P**
Water content (max.) (%)	13.0	13.0
Protein (%)	22.0-23.0	21.5-23
Crude fiber (max.) (%)	5.0	4.0
Fat (min.) (%)	5.0	8.0
Ash (max.) (%)	7.0	6.5
Ca (%)	0.9	0.9-1.2
P (%)	0.6	0.7-1.0
EM Kcal/kg	3020-3120	3000-3100

Source: *PT. Charoen Pokphand Indonesia **PT. Cj Cheiljedang Feed Lampung

Table 2. Average live weight of broiler chickens at the end of the study

Treatment	Live weight (g)
P0	1315.00±38.50 ^a
P1	1574.20±37.20 ^b
P2	1619.30±65.60 ^b

^{a, b}Different superscripts within the same column indicate significant different (P<0.05), P0= Without the addition of propionic acid), P1= With the addition of propionic acid of 0.5%), P2= With the addition of propionic acid of 0.75%

significant difference, then a further test is carried out using the Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Effect of Treatment on Live Weight

The effect of the addition of propionic acid on the live weight of broilers is presented in Table 2. The results of the analysis of variance showed that the addition of propionic acid had a significant effect ($P < 0.05$) on the live weight of broilers. This was because the addition of propionic acid in the feed can increase feed consumption, thereby increased live weight at the end of the study. The feed consumption in this study was 73.94 g/chicken/day, 75.64 g/chicken/day and 75.87 g/chicken/day in P0, P1, and P2, respectively. The increase in feed consumption was caused by propionic acid effect to improve the performance of digestive enzymes, thus shortening the time to digest food substances. Gautier (2002) stated that organic acids can increase the rate of feed flow so that gastric emptying is faster and causes an increase in feed consumption during. Rasyaf (2011) reported that feed consumption is an illustration of the entry of a number of nutrient elements into the body of broiler chickens.

Further test results showed that the live weight of broiler chickens in P0 (control) was significantly different ($P < 0.05$) from those in group P1 and P2. This was because the addition of propionic acid in the feed was able to maintain the pH of the digestive tract to remain acidic. Acidic pH conditions lead to the death of pathogenic bacteria as pathogenic bacteria cannot withstand these acidic conditions. A low pH value increased the growth of good bacteria in the digestive tract. Emma *et al.* (2009) reported that giving lime juice containing citric acid at the level of 0.4% and 0.8% was able to increase the number of lactic acid bacteria and reduce *Salmonella* sp. in the digestive tract of broiler chickens and is able to maintain an acidic pH in the digestive tract. According to Mabelebele *et al.* (2014), the pH of the digestive tract of broiler chickens ranges from 3.47 (gizzard) to 6.43 (small intestine). Saputra *et al.* (2013) reported that organic acids are able to maintain the pH of the digestive tract (cache, ventriculus and intestines), that in turn suppress the

growth of pathogenic bacteria and increase lactic acid bacteria which contribute to the digestive process, so that protein utilization is good for absorption of nutrients that will be used for form muscle tissue. This is in line with the opinion of Naseri *et al.* (2012) that organic acids act as growth promoters capable of suppressing the growth of acid-intolerant bacteria such as *Escherichia coli*, *Salmonella* spp., and *Clostridium perfringens*. According to Huyghebaert (2005), reducing the population of pathogenic bacteria reduces the competition for nutrient use against the host and increases the population of lactic acid bacteria. Lactic acid bacteria help the digestive protease enzyme in the intestine, maintain intestinal health, and help absorption of nutrients (Islam *et al.*, 2008).

Treatment P1 (addition of 0.5% propionic acid) was not significantly different ($P > 0.05$) from P2 (addition of propionic acid 0.75%) to the percentage of live weight. This is because the addition of 0.5% propionic acid is able to maintain the pH of the digestive tract, allowing the digestive process to run well. Afsharmanesh and Porreza (2005) stated that the addition of organic acids can maintain the acidic pH of the digestive tract thereby increasing the activity of the pepsin enzyme. Pepsin enzyme functions to break down protein into amino acids and is absorbed by the body, so that the process of forming muscle tissue is good and can increase body weight gain in broiler chickens. This is in line with the research results of Radhiyani *et al.* (2017) which reported that the addition of 0.75% acetic acid in the feed resulted in better body weight compared to the control groups. Islam *et al.* (2008) stated that the administration of 0.5% citric acid showed the best final body weight results.

Effect of Treatment on Percentage of Internal Organs of Broiler Chickens

The average effect of the addition of propionic acid on the percentage of internal organs (spleen, gizzard, and liver) of broilers can be seen in Table 3. The results of the analysis of variance showed that the addition of propionic acid had no significant effect ($P > 0.05$) on the percentage of internal organs of the broilers, indicating that propionic acid did not have any negative impact on the internal organs of broilers. This is because propionic acid's ability to suppress the growth of

Table 3. Average percentage of physiological organs (spleen, gizzard, and liver)

Treatment	Spleen percentage (%)	Gizzard percentage (%)	Liver percentage
P0	0.10±0.04	1.67±0.39	2.54±0.34
P1	0.14±0.02	1.59±0.22	2.24±0.36
P2	0.15±0.03	1.47±0.17	2.42±0.26

P0= Without the addition of propionic acid), P1= With the addition of propionic acid of 0.5%), P2= With the addition of propionic acid of 0.75%

Table 4. Length of the small intestine

Treatment	Length (cm)		
	Duodenum	Jejunum	Ileum
P0	28.67±2.25	70.50±8.36	56.67±6.19
P1	29.33±2.87	71.83±9.87	57.83±6.76
P2	31.50±5.39	77.83±7.39	59.17±6.43

P0= Without the addition of propionic acid), P1= With the addition of propionic acid of 0.5%), P2= With the addition of propionic acid of 0.75%

pathogenic or toxic microorganisms so that the performance of internal organs becomes normal and the metabolism runs well. According to Jamilah *et al.* (2014), the addition of organic acids is able to improve the immunity of livestock so that macrophages function properly and are able to kill antigens before they reach the bloodstream to potentially inhibit the work of internal organs. The good immunity of broiler chickens causes less protein to be used for tissue repair and antibody formation, so that protein will be deposited into the tissue for the better growth. Amrullah (2004) stated that health conditions, absorption and slow flow rates will affect the size of the digestive tract; it becomes heavier, longer, and thicker.

The average percentage of spleen weight in this study is still in the normal range (0.10%-0.15%). According to Resnawati (2010), normal spleen weight in broilers ranges from 0.08 to 0.18% of live weight, whereas according to Hermana *et al.* (2008) the spleen weight percentage of broiler chickens aged five weeks ranges from 0.09%-0.14% and the spleen percentage of broiler chickens aged 35 days with the provision of various acidifiers and probiotics ranges from 0.11 to 0.13% (Kermanshahi *et al.*, 2017). The percentage of broiler gizzard weight found in this study ranged from 1.47%-1.67%, and is still in the normal range. According to Ramli *et al.* (2008), the percentage of gizzard weight ranges from 1.88%-2.23% of live weight whereas Kokoszynski *et al.* (2017) reported that broiler gizzard weight ranges from 1.20%-1.30%. The gizzard weight obtained, however, is lower than that reported by Malik *et al.* (2016) in broiler chickens fed with 0.05% probiotic, 0.20% acidifier, and their combination, namely 2.37% to 2.56%. Ali *et al.* (2018) also reported higher gizzard weight (2.06%) in broilers fed with organic acids as a substitute for antibiotics.

The average percentage of broiler liver weight in this study ranged from 2.24%-2.54%. According to Khotimah (2002), the normal percentage of liver weight ranges from 2.15%-2.59%. Another study reported that the average percentage of broiler liver is 2.61-2.78% of the live weight whereas the results of study performed by Natsir (2008) showed that the relative liver weight of broiler chicken fed with a combination of citric acid and lactic acid was 2.00-2.81%.

Effect of Treatment on Small Intestine Length

The effect of the addition of propionic acid in feed on the length of the small intestine of broiler chickens is presented in Table 4. The results of the analysis of variance showed that the addition of propionic acid up to 0.75% had no significant effect ($P>0.05$) on the length of the duodenum, jejunum, and ileum. This indicates that propionic acid does not have a negative impact on the small intestine. According to Zunita *et al.* (2004), the addition of organic acids in animal feed can maintain gut performance as it creates a harmonious environment for the development of beneficial microflora. A balanced microflora condition causes the formation of a good defense system in the intestinal lumen (Jeppsson *et al.*, 2004).

The average length of duodenum observed in this study ranged from 28.67-31.50 cm, while the jejunum length ranged from 70.50-77.83 cm, and the ileum ranged from 56.67-59.17 cm. The total length of the intestine in this study ranged from 155.84 to 168.50 cm. Mabelebele *et al.* (2014) reported that the intestinal length of the broiler chicken with Ross strain 308 was 163.10 cm (duodenum 31.90 cm, jejunum 60.60 cm, and ileum 70.60 cm). The length of the small intestine found in this study was longer compared to the results of reported by Fernandes *et al.* (2014) which observed that the length of small intestine of broiler chickens aged 42 days fed with several combinations of organic acids is 141.37 cm with a duodenal length 30.25 cm, jejunum 55.87 cm and ileum 55.25 cm. Meanwhile, the results of research by Adil *et al.* (2011) reported a longer small intestine in broiler chickens fed with several types of organic acids than those fed without organic acids supplementation. Rehman *et al.* (2016) reported that broilers whose feed was supplemented with acetic acid up to 0.3% had a longer small intestine (1.43%-5.44%) compared broiler chickens whose feed without acetic acid supplementation. This increase is related to the increase in the length and width of the intestinal villi (Sabour *et al.*, 2019).

CONCLUSION

It can be concluded that the addition of propionic acid in poultry feed up to 0.75% is able to increase the percentage of live weight, but does not have a negative impact on the condition of the internal organs and the length of the small intestine of broiler chickens.

REFERENCES

- Adil, S., T. Banday, A.G. Bhat, M. Salahudin, M. Raquib, and S. Shanaz. 2011. Response of broiler chicken to dietary supplementation of organic acids. *J. Cent. Europ. Agric.* 12(3):489-508.
- Afsharmanesh, M. and J. Porreza. 2005. Effects of calcium, citric acid, ascorbic acid, vitamin D on the efficacy of microbial phytase in broiler starters fed wheat-based diets: performance, bone mineralization and ileal digestibility. *Int. J. Poultry Sci.* 4:418-424.
- Ali, N., S. Alkassar, and S. Alkassar. 2018. The effect of using organic acid as an alternative to antibiotics drugs on productive and physiological performance of broilers Ross- 308. *Res. Art. Cross Mark.* 6(9):359-366.
- Amrullah, I.K. 2004. *Nutrisi Ayam Broiler*. Seri Beternak Mandiri. Lembaga Satu Gunung Budi, Bogor.
- Daud, M. 2005. Performan ayam pedaging yang diberi probiotik dan prebiotik dalam ransum. *J. Ilmu Ternak.* 5(2):75-79.
- Emma, W.M.S.M., O. Sjojfan, Achmanu, and E. Widodo. 2009. Efek ekstrak jeruk nipis terhadap jumlah koloni bakteri asam laktat, *E. coli* dan *salmonella* dalam ileum ayam pedaging. *J. Ilmu Ilmu Peternakan.* 19:28-34.
- Fernandes, B.C.S., M.R.F.B. Martins, A.A. Mendes, E.L. Milbradt, C. Sanfelice, B.B. Martins, E.F. Aguiar, and C. Bresne. 2014. Intestinal integrity and performance of broiler chickens fed a probiotic, a prebiotic, or an organic acid. *Braz. J. Poultry Sci.* 16(4):417-424.
- Gauthier, R. 2002. *Intestinal Health, the Key to Productivity (The Case of Organic Acids)*. Precongreso Cientifico Avicola IASA. Puerto Vallarta, Jal. Mexico.
- Hardy, B. 2003. *Nutraceutical Concepts of Gut Health in Pigs*. NutriVicion Inc. Fairmont, Minnesota.
- Hermana, W., D.I. Puspitasari, K.G. Wiryawan, and S. Suharti. 2008. Pemberian tepung daun salam (*Syzygium polyanthum* (Wight)

- Walp.) dalam ransum sebagai bahan antibakteri *Escherichia coli* terhadap organ dalam ayam broiler. **Media Peternakan**. 31(1):63-70.
- Huyghebaert, G. 2005. Alternatives for Antibiotics in Poultry. **Proceedings of the 3rd Mid-Atlantic Nutrition Conference**. Timonium, Maryland:36-57.
- Islam M.Z., H.Z. Khandaker, D.S. Chowdhury, and K.M.S. Islam. 2008. Effect of citric acid and acetic acid on the performance of broilers. **J. Bangladesh Agric. Univ.** 6(2):315-320.
- Jamilah., N. Suthama, and L.D. Mahfudz. 2014. Pengaruh penambahan jeruk nipis sebagai acidifier pada pakan step down terhadap kondisi usus halus ayam pedaging. **J. Ilmu Teknologi Peternakan**. 3(2):90-95.
- Jeppsson, B., P. Mongell, D. Adawi, and G. Molin. 2004. Bacterial translocation: Impact of probiotics. **Scandinavian J. Nutr.** 48:37-41.
- Julendra, H., Zuprizal, dan Supadmo. 2010. Penggunaan cacing tanah (*Lumbricus rubellus*) sebagai aditif pakan terhadap penampilan produksi ayam pedaging, profil darah dan pencernaan protein. **Bull. Peternakan**. 34(1):21-29.
- Kermanshahi, H., R.M. Heravi, A. Attar, A.R. Pour, E. Bayat, M.H. Zadeh, A. Daneshmand, and S.A. Ibrahim. 2017. Effects of acidified yeast and whey powder on performance, organ weights, intestinal microflora, and gut morphology of male broilers. **Brazilian J. Poultry Sci.** 19(2):309-316.
- Khosravi, A., F. Boldaji, B. Dastar, and S. Hasani. 2012. Comparison of broiler performance and carcass parameters when fed diets containing a probiotic, an organic acid or antibiotic growth promoter. **Asian J. Anim. Vet. Adv.** 7:318-325.
- Khotimah, K. 2002. Pengaruh Ekstrak Jeruk Nipis dan Metode Pengolahan pada Kualitas Daging Ayam Pedaging. <http://digilib.si.itb.ac.id/print.php?id=jiptumm-gdl-Res-2002-ir-5311- jeruk>.
- Kokoszynski D., Z. Bernacki, M. Saleh, K. Stęczyński, and M. Binkowska. 2017. Body Conformation and internal organs characteristics of different commercial broiler lines. **Braz. J. Poultry Sci.** 19 (1):047-052.
- Lu, J., U. Idris, B. Harmon, C. Hofacre, J. Maurer, and M.D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. **Appl. Environ. Microbiol.** 69(11):6816-6824.
- Mabelebele, M., J.O. Alabi, W.J. Ng'ambi, D. Norris, and M.M. Ginindza. 2014. Comparison of gastrointestinal tracts and pH value of digestive organs of Ross 308 broiler and indigenous Venda chickens fed the same diet. **Asian J. Anim. Vet. Adv.** 9(1):71-76.
- Malik, H.E.E., R.H.H. Hafzalla, O.H.A. Ali, M.M.O. Elhassan, B.M.A. Dousa, M. Ali, and M.E. Khalid. 2016. Effect of probiotics and acidifiers on carcass yield, internal organs, cuts and meat to bone ratio of broiler chicken. **J. Agric. Vet. Sci.** 9(12):18-23.
- Naseri K.G., S. Rahimi, and P. Khaki. 2012. Comparison of the effects of probiotic, organic acid and medicinal plant on *Campylobacter jejuni* challenged broiler chickens. **J. Agric. Sci.** 14:1485-1496.
- Natsir, M.H. 2008. Pengaruh penggunaan kombinasi asam sitrat dan asam laktat cair terenkapsulasi sebagai aditif pakan terhadap persentase karkas dan berat organ dalam ayam pedaging. **J. Nutrisi Makanan Ternak**. 3(2):17-22.
- Papatsiros, V.G. and C. Billinis. 2014. The Prophylactic Use of Acidifier as Antibacterial Agent in Swine. In **Antimicrobial Agents**. Varaprasad, B. (Ed.). In Tech, Croatia.
- Radhiyani U.A., N. Suthama, and I. Mangisah. 2017. Pengaruh penambahan asam asetat pada ransum dengan level protein berbeda terhadap retensi kalsium dan massa protein daging pada ayam broiler. **Agromedia**. 35(1):21-27.
- Ramli, N., D.M. Suci, S. Sunanto, C. Nugraheni, A. Yulifah, and A. Sofyan. 2008. Performan ayam broiler yang diberi ransum mengandung potasium diformat sebagai pengganti flavomycin. **Agripet**. 8:1-8.
- Rasyaf, M. 2011. **Panduan Beternak Ayam Pedaging**. Penebar Swadaya, Jakarta.
- Rehman, Z.U., A.U. Haq, N. Akram, M.E. Abd El-Hack, M. Saeed, S.U. Rehman, C. Meng, M. Alagawany, M. Sayab, M.K. Dhama, and C. Ding. 2016. Growth performance, intestinal histomorphology, blood hematology and serum metabolites of broilers chickens fed diet supplemented with graded levels of acetic acid. **Int. J. Pharmacol.** 12:874-883.
- Resnawati, H. 2010. Bobot Organ-Organ Tubuh pada Ayam Pedaging yang Diberi Pakan Mengandung Minyak Biji Saga (*Adenanthera pavonina* L.). **Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner**. Pusat Penelitian dan Pengembangan Peternakan. Bogor:670-673.
- Rimbawanto, E.A., N. Iriyanti, and B. Hartoyo. 2019. Bobot dan Panjang Usus Halus serta Bobot Organ Aksesoris Ayam Broiler dengan Pemberian Berbagai Jenis Acidifier. **Prosiding Seminar Nasional Pengembangan Sumber Daya Perdesaan dan Kearifan Lokal Berkelanjutan IX**. Purwokerto:105-112.
- Roth, F.X. and M. Kirchgessener. 2003. **The Role of Formic Acid in Animal Nutrition**. Institute for Animal Nutrition and Physiology, Technical University of Munich, Munich
- Sabour, S., S.A. Tabeidian, and G. Sadeghy. 2019. Dietary organic acid and fiber sources affect performance, intestinal morphology, immune responses and gut microflora in broilers. **J. Anim. Nutr.** 5:156-162.
- Saputra, W.Y., L.D. Mahfudz, and N. Suthama. 2013. Pemberian pakan single *stepdown* dengan penambahan asam sitrat sebagai acidifier terhadap performa pertumbuhan broiler. **Anim. Agric.** 2(3):61-72.
- Zunita Z., Y.M. Gob, N. Jefri, M.H. Latiffah, and S.K. Ang. 2004. In vitro study on the antimicrobial activities of Orgacids™ against some important bacteria. **International Conference of the Institute for Tropical Veterinary Medicine**. Belgium:340-341.