p-ISSN 1410-5659 e-ISSN 2621-5144 Jurnal Ilmu Ternak Universitas Padjadjaran, Desember 2023, 23(2):170-177
Published by: DOI: 10.24198/jit.v23i2.46212
Unpad Press Available online at http://jurnal.unpad.ac.id/jurnalilmuternak

UTILIZATION OF KEPEL (*STELECHOCARPUS BURAHOL*) LEAF EXTRACT AS A FEED ADDITIVE IN BROILER CHICKEN

Adi Ageng Mustawa[1, a], Tuti Widjastuti[1], Denny Rusmana[1]
[1]Faculty of Animal Husbandry, Padjadjaran University
Jln. Ir. Soekarno Km 21 Jatinangor, Sumedang 45363.
[a]*email*: adimustawa@gmail.com

Abstract
This study aims to determine the best level of Kepel leaf extract (*Stelechocarpus burahol*) used in feeds based on the quantity of *Escherichia coli* in excreta, ileal villi, protein digestibility, ration consumption, and body weight gain (BWG) of 100 broiler chickens. The research was conducted experimentally using a completely randomized design (CRD), four levels of Kepel leaf extract in rations repeated five times. The Kepel leaf extract (EDK) levels used consisted of P_0 = basal ration, $P_1 = P_0$ + 0.15% EDK, $P_2 = P_0$ + 0.30% EDK and $P_3 = P_0$ + 0.45% EDK. Parameters observed were the quantity of *E. coli* in the excreta, ileal villi, protein digestibility, feed consumption, and PBB of broiler chickens. Data were processed using analysis of variance, and if the results were significantly different, then Duncan's test was carried out. The results showed that adding a 0.30% Kepel leaf extract to the feed significantly affects ($p<0.01$) the number and width of ileal villi and increases protein digestibility. However, it had no significant effect ($p>0.05$) on the number of *E. coli* on excreta, villi height, ration consumption, and BWG.
Keywords: kepel leaf extract, broiler chicken, ileal villi, digestibility, performance

PEMANFAATAN EKSTRAK DAUN KEPEL (STELECHOCARPUS BURAHOL) SEBAGAI FEED ADDITIVE DALAM RANSUM AYAM BROILER

Abstrak
Penelitian ini bertujuan untuk mengetahui taraf terbaik penggunaan ekstrak daun kepel (Stelechocarpus burahol) dalam ransum terhadap jumlah Escherichia coli pada ekskreta, vili ileum, kecernaan protein, konsumsi ransum dan pertambahan bobot badan (PBB) pada 100 ekor ayam broiler. Penelitian dilakukan secara eksperimental menggunakan Rancangan Acak Lengkap (RAL), 4 taraf ekstrak daun kepel dalam ransum diulang sebanyak 5 kali. Taraf ekstrak daun kepel (EDK) yang digunakan terdiri atas T0 = pakan basal, T1 = T0 + 0,15% EDK, T2 = T0 + 0,30% EDK dan T3 = T0 + 0,45% EDK. Parameter yang diamati adalah jumlah E. coli pada ekskreta, vili ileum, kecernaan protein, konsumsi ransum dan PBB ayam broiler. Pengolahan data menggunakan analisis sidik ragam dan jika hasil berbeda nyata, maka selanjutnya dilakukan Uji Duncan. Hasil penelitian menunjukan bahwa taraf 0,30% ekstrak daun kepel dalam ransum berpengaruh sangat nyata (p<0,01) terhadap jumlah dan lebar vili ileum serta kecernaan protein, namun tidak berpengaruh nyata (p>0,05) terhadap jumlah E. coli pada ekskreta, tinggi vili, konsumsi ransum dan PBB ayam broiler.
Kata kunci: ekstrak daun kepel, ayam broiler, vili ileum, kecernaan, performa

INTRODUCTION

The nutritional content of feed significantly influences the growth and development of broiler chickens. Additives are commonly used in feed to enhance metabolic efficiency and nutrient absorption. The small intestine plays a central role in nutrient absorption, where a greater number of villi in the small intestine provides a wider surface wall area of the small intestine to expedite and increase the absorption of nutrients in the animal's body (Awad et al., 2008). Optimal nutrient absorption depends on the health and condition of intestine. Intestinal health is affected by the microbial population contained therein (Halimatunnisroh et al., 2017).

Escherichia coli belongs to the group of gram-negative pathogenic bacteria *Enterobacteriaceae*. These bacteria, when present in the small intestine, can impact the health and growth of broiler chickens. Pathogenic bacteria can damage the intestinal wall, impending the growth and nutrient absorption of villi, and causes diarrhea by inhibiting fluid reabsorption, resulting in watery excreta. This loss of absorbed fluids weakens the body and reduces appetite (Blowey & Weaver, 2011). Pathogenic bacteria can also impact the crude protein analysis by reducing calculated crude protein digestibility values (Sari et al., 2014). The population of pathogenic bacteria increases in the absence of antibacterial compounds to inhibit their development. An overpopulation of pathogenic bacteria in the digestive tract can lead to depression, diarrhea, reduced digestibility, and even death.

The use of growth-inducing chemical antibiotics in feeds has been banned due to concerns about antimicrobial residues that can be harmful to consumers' health. These chemical antibiotics can be replaced by natural phytopharmaca containing antibacterial compounds (Djojoningrat, 2006). One such natural antimicrobial source is the leaves of the Kepel fruit plant (*Stelechocarpus burahol*), a plant originating from the Special Region of Yogyakarta. Kepel leaf extract are known to contain flavonoid compounds, saponins, tannins, polyphenols, and isoflavones (National Food and Drug Administration, 2011). LC-MS analysis test has confirmed the presence of proanthocyanidin compound (27.59%) in extracted *Kepel* leaves (procedure involving maceration of 2 days of immersion in 70% ethanol) (Nuryana, 2020; Puslabfor Polri, 2019).

Proanthocyanidins, compounds of the flavonoid class (condensed tannins), reduce the population of enterobacteria by binding to phenol, indole, skatol, and cresol compounds that foster enterobacterial growth in the intestine (Yamakoshi et al., 2001). Proanthocyanidins, commonly found in grape seed extract, is reported to disrupt the metabolism of harmful microbes and exhibit antibacterial activity against several bacteria, including: *S. aureus, S. pneumoniae, S. epidermidis, St. pyogenes, E. coli, H. influenzae, P. aeruginosa, En. faecalis, Mr. casilliflavus* (Mayer et al., 2008). The addition of *Kepel* leaf extract to an appropriate level, functioning as a natural antibacterial in broiler diets, is believed to interfere with the growth or inhibit harmful bacteria in the chickens' intestines. A low number of pathogenic bacteria in the intestine is expected to optimize the absorption of nutrients by the intestinal villi, consequently increasing protein digestibility accompanied and increasing body weight.

MATERIALS AND METHODS

A total of 100 one-day-old (DOC) Cobb-strained broiler chicks were divided into 20 experimental unit plots. The DOCs were placed randomly within five individuals/per pen within each experimental unit. Each experimental animal was given a tag on its wing to facilitate individual identification during observation. The Kepel leaf extraction was carried out in Central Lab, Padjadjaran University, Sumedang. Dry Kepel leaves were extracted using the maceration method with 70% ethanol liquid for two days. The final step involved evaporating the liquid at 40 ^{0}C until a concentrated extract was obtained.

The in-vivo testing of the Kepel leaf extract product in broiler diets was conducted through a 30-day feeding regimen at the Print-G Research, Nano Biomaterials Laboratory, Faculty of Math and Science, Padjadjaran University. The experiment was designed as a Completely Randomized Design (CRD), and consisted of 4 levels of Kepel leaf extract (EDK) supplementation in feeds, each with five replications. The treatments were labeled as follows: T0 for basal diet, T1 for basal diet + 0.15% EDK, T2 for basal diet + 0.30% EDK, and T3 for basal diet + 0.45% EDK. The nutritional content of the basal feed used in this study is presented in Table 1.

Parameters observed included the number of *E. coli* bacteria in excreta, ileal villi, ration consumption, protein digestibility, and body weight gain of broiler chickens. Sampling was conducted on the last day of maintenance period. The quantification of *E. coli* bacteria in excreta was calculated using the Total Plate Count (TPC) test method using *E. coli Mac Concay* Agar at the Central Laboratory of Padjadjaran University.

The procedure for measuring the number of ileal villi began with slaughtering 20 sample of chickens, collecting the ileum (approximately 2 cm before the large intestine), and rinsing it with 0.9% NaCl solution. The collected ileal samples were then immersed in a test tube containing a fixative solution (Bouin). Subsequently, the samples were examined through a microscope and counted using ImageJ software at the Laboratory of

Biosystem Microtechnics, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

The method used to measure the protein digestibility of the ration added with Kepel leaf extract was by using lignin as an indicator. The protein and lignin percentage in broiler feeds and excreta were tested at the NTR and KMT Laboratories, Faculty of Animal Husbandry, Padjadjaran University. The protein digestibility value was calculated based on the method described by Schneider and Flatt (1975) as follows:

Protein Digestibility =

$$100\% - 100 \left| \frac{\%\ Lignin\ Ransum}{\%\ Lignin\ Feses} \mathrm{x} \frac{\%\ Protein\ Feses}{\%\ Protein\ Ransum} \right|$$

Feed consumption was determined by weighing the feed provided and subtracting the weight of the feed remaining unconsumed. Broiler chicken weight gain was calculated based on the difference in body weight before and after treatment.

RESULTS AND DISCUSSION

Escherichia coli in excreta

The amount of *Escherichia coli* (*E. coli*) in chicken's excreta between treatments is presented in Table 2. The results indicate that treatment T3 had the highest *E. coli* count (241.76 x 10^4 CFU/gr), while the lowest *E. coli* count was observed in treatment T1 (106.64 CFU/gr). However, the addition of Kepel leaf extract did not have a significant effect on increasing the *E. coli* count in the excreta of broiler chickens.

One potential factor that may have influenced these results is that the chickens treated *Kepel* leaf extract were housed in the same room as chickens without treatment. This proximity could have allowed the transmission of *E. coli* through the air. Furthermore, the excreta used for testing was mixed with the husk from the cage floor, which may have led to contamination by various environmental conditions, including *E. coli*. These conditions could have contributed to the less accurate results when assessing the *Kepel* leaf extract in broiler feeds. As mentioned by Lusandika (2017), pathogenic bacteria like *Coliform* and *E. coli* can thrive in chicken coops with infrequently cleaned drinking places and damp, lumpy litter.

Kepel leaf extract contains saponins, tannins, flavonoids, polyphenols, and isoflavones (BPOM RI, 2011). Nuryana (2020) demonstrated that condensed tannin compounds (proanthocyanidins), found in Kepel leaf extract, provide resistance against toxins produced by *E. coli* bacteria. Low concentrations of tannins can inhibit bacterial growth, whereas at high concentrations, they form stable bonds with bacterial proteins by aggregating the bacterial cytoplasm (Robinson, 1995; Wiryawan et al., 2007). Flavonoids are phenolic compounds that can hinder the action of enzymes from interfering with bacterial metabolic activity by binding to bacterial proteins. Saponins are compounds that can cause hemolysis in bacterial cells by increasing the permeability of bacterial cell membranes (Robinson, 1995; Wiryawan et al., 2007).

Amalia et al. (2020) found that adding Kepel leaf extract at a 0,15% concentration in the ration gave an optimal effect on reducing the number of Enterobacteriaceae bacteria to 5,0 x 104 CFU/Unit, and significantly reduced the number of gram-negative bacteria *Enterobacteriaceae* in the ileum digesta of broiler chickens. *E. coli* belongs to the *Enterobacteriaceae group* of bacteria, a large and heterogeneous group of gram-negative rods. *E. coli* is facultative anaerobic and typically resides in the digestive tract. Most of the *Enterobacteriaceae* bacteria are known to cause gastrointestinal tract infections, therefore, the presence of *Enterobacteriaceae* bacteria in the small intestine can have adverse effects on the health and growth of broiler chickens (Brooks et al., 2008).

The results of this study provide a more accurate reference because it minimizes intervention from the environmental conditions within the pens. The compounds present in Kepel leaf extract can function as antibacterials against the gram-negative bacteria group *Enterobacteriaceae* in the ileum of broiler chickens. While these compounds may not specifically target the *E. coli* population, they have a beneficial impact on overall gut health and bacterial balance in the broiler chickens.

Ileum Villi

Table 3 shows the impact of different Kepel leaf extract treatments on the number, number, height, and width of ileal villi in broiler chickens. No addition of Kepel leaf extract or T0 exhibited the lowest average number (29) and width (88.15 µm) of ileal villi. On the other hand, treatment T2 showed the best average number (39.2) and height (476.56 µm) of ileal villi. The best width of ileal villi (106.33 µm) was found in treatment T3. These results indicate that the addition of Kepel leaf extract at a level of 0.30% has a highly significant effect on the number of ileal villi. The addition of 0.45% Kepel leaf extract significantly affected the width of the ileal villi, but not the height of the ileal villi in broiler chickens.

The population of pathogenic bacteria increases when there are no antibacterial compounds to inhibit its growth. An excess population of pathogenic bacteria in the digestive tract can lead to various health issues, including depression, diarrhea, reduced digestibility, and death. Pathogenic bacteria infect the digestive tract by attaching to enterocyte cells through structures like pili (fimbriae). They then proliferate and colonize the intestinal mucosa, increasing in number and causing lesions. This, in turn, results in thickening and damage to the intestinal wall, interfering with the villi growth and the nutrients absorption (Biowey & Weaver, 2003).

Kepel leaf extract, containing flavonoids and saponins, has the potential in inhibiting pathogenic bacteria activity in the gut. Flavonoids, as phenolic compounds, can inhibit the work of enzymes to interfere with bacterial metabolic activity by binding to bacterial proteins, while saponins can hemolyze bacterial cells by increasing the permeability of bacterial cell membranes (Robinson, 1995; Wiryawan et al., 2007). These mechanisms likely contribute to the observed effects on the ileal villi and gut health in broiler chickens.

The presence of proanthocyanidins (condensed tannins) in *Kepel* leaf extract is believed to inhibit the growth of harmful bacteria found in the small intestine of broiler chickens. A balanced number of pathogenic and non-pathogenic bacteria in the small intestine will result in healthy minor intestine conditions, which, in turn, supports the growth of broiler chicken ileal villi. Amalia et al. (2020) stated that the proanthocyanidin compounds found in *Kepel* leaf extract could act as antibacterial agents for *Enterobacteriaceae* in the ileum of broiler chickens. The mechanism by which antibacterial compounds inhibits bacterial growth involves damaging the bacterial cell wall, resulting in cell rupture. They alter the permeability of the cytoplasmic membrane, which causes leakage of nutrients inside the cell and denatures cell proteins, preventing nutrients in the cells to trigger bacterial growth. This disrupts the bacterial cell's metabolic system by inhibiting intracellular enzymes that work as catalysts for cellular metabolism (Amalia, 2020; Bambang et al., 2014). Moreover, Nuryana (2020) stated that proanthocyanidins provide a protective effect on the small intestine walls, guarding them against toxins produced by gram-negative pathogenic bacteria.

T0
(Without Treatment)

T2
+0.30% Kepel Leaf Extract)

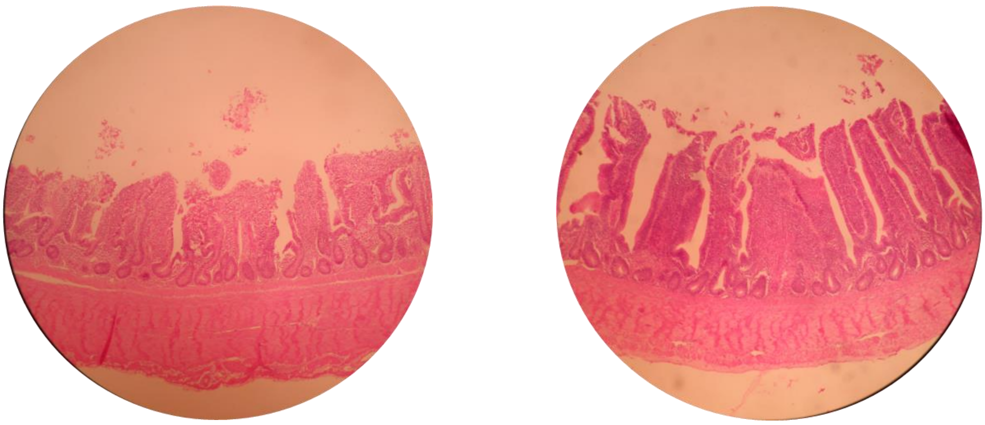

Figure 3. Ileum Morphometric Histological Image of Broiler Chickens

Protein Digestibility

Protein digestibility refers to the efficiency with which the digestive system breaks down proteins from the diet and absorbs them for utilization in the body. Several factors can influence feed digestibility, including the form or composition of the feed, the species of the animal, feed composition, feed quantity, ambient temperature, and the age of the livestock (Ranhjan, 1980; Sutrisno & Suthama, 2013). T0 treatment (without adding *Kepel* leaf extract) had the lowest protein digestibility percentage (48.63%), and the T2 treatment resulted in the highest percentage of protein digestibility (67.03%) (Table 4). These results indicate that the addition of *Kepel* leaf extract at a level of 0.30% had a very significant effect on protein digestibility in broiler chickens. The findings of the study indicate that the increase in the number, height, and width of the ileal villi in the digestive tract of broiler chickens, attributed to the addition of Kepel leaf extract, could be a key factor in the observed increase in protein digestibility. This is consistent with the study by Awad et al., (2008) that having a greater number of intestinal epithelial folds, an increased surface area, and more villi and microvilli leads to a wider area for nutrient absorption, ultimately enhancing the digestion and absorption of nutrients transport system throughout the body.

The balance of bacteria in the digestive tract plays a critical role in the overall health and digestive function of poultry. An excess population of pathogenic bacteria in the digestive tract is reported to reduce digestibility. The balance of good bacteria in the intestine positively affects the health and function of the intestine and increases protein digestibility. Protein digestion in poultry takes place in the proventriculus by the enzyme pepsin, and pancreatic enzyme secretion is stimulated by the cholecystokinin hormone, secreted from the small intestine mucosa (Hidayat et al., 2010). Therefore, the condition of a healthy small intestine is closely related to the ability of digestive function and absorption of nutrients. Mountzouris et al. (2010) also state that the balance of bacteria in the digestive tract can improve the function and health of the digestive tract, thereby improving the digestive process nutrients, especially protein, and calcium.

Consumption of Rations and BWG

Table 5 presents the impact of Kepel leaf extract supplementation on broiler body weight gain (BWG) and feed consumption. There was a slight increase in body weight gain in group the groups receiving *Kepel* leaf extract supplementation, but it was not statistically significant. The highest average body weight gain (BWG) was observed in T1 treatment (41.01 gram), while the T0 treatment, without the addition of *Kepel* leaf extract, had the lowest BWG (35.36 g/head).

Feed consumption is a benchmark for assessing livestock's food intake and is closely related to increased body weight. Higher feed consumption is generally expected to be directly proportional to increased body weight, although it is influenced by livestock genetics, the type of feed given, feed additives, maintenance management, and environmental temperature (James, 2004). The standard average ration consumption for MB-202 broiler in the fourth week, according to Japfa Comfeed Indonesia (2012), is 2,180 g/head, while in group without Kepel supplementation (T0). This group consumed 2,031.7 g/head of feed at 30 days of age. However, there was a reduction in ration consumption in treatment groups with Kepel leaf extract addition (T1, T2, and T3).

BPOM RI (2011) reported that Kepel leaf extract contains saponins, a bitter chemical compound in plants that can produce foam when shaken vigorously in a solution. High saponin content in feed may lead to a bitter taste and reduced palatability, which can subsequently lower feed intake and overall livestock productivity. According to Jayanegara et al. (2019), 0.1–0.3% of saponin compounds in poultry rations can adversely impact feed consumption, feed efficiency, poultry growth and egg production in laying hens. In contrast, as found in a study by Amananti (2017), 0.9% triterpenoid saponins in chick rations can lead to increased body weight, fat digestibility, and cholesterol excretion. However, it also results in reduced absorption of vitamin D, A and feed consumption. In this experiment, the addition of burahol leaf extract in rations containing saponins appears to have contributed to the non-significant results on broiler body weight gain. Decreased palatability affects feed consumption, leading to a reduction in nutrient intake for growth, and resulted in decreased broiler body weight.

Table 1. Nutrient Content of Basal Ration

No.	Nutrients	Content
1	Metabolic Energy (kcal/kg)	3,106
2	Crude Protein (%)	23,97
3	Calcium (%)	0.90
4	Phosphorus (%)	0.50
5	Crude Fat (%)	4.50
6	Crude Fiber (%)	3.84
7	Methionine (%)	0.56
8	Lysine (%)	1.33

Note: Results of Laboratory Analysis of Ruminant Animal Nutrition and Animal Feed Chemistry, Faculty of Animal Husbandry, Padjadjaran University;

Table 2. Number of Excreted *E. Coli Bacteria*

Parameter	Treatment			
	T0	T1	T2	T3
E. Coli (10^4 CFU/gr)	137.52	106,64	230.36	241.76

Description: T0: basal ration; T1: basal ration + 0.15% Kepel leaf extract; T2: basal ration + 0.30% Kepel leaf extract; T3: basal ration + 0.45% Kepel leaf extract.

Table 3. Mean Number of Ileal Villi, Height of Ileal Villi, Width of Ileal Villi

Parameter	Treatment			
	T0	T1	T2	T3
Number of Ileum Villi	29[a]	32.8[b]	39.2[c]	32[b]
Ileum Villi Height (µm)	461.14	468.08	476.56	412.01
Ileum Villi Width (µm)	88.15[a]	100.21[b]	105.84[c]	106.33c

Note: Different superscripts in the same column show very significant differences (P<0.01);
T0: basal ration; T1: basal ration + 0.15% Kepel leaf extract;
T2: basal ration + 0.30% Kepel leaf extract; T3: basal ration + 0.45% Kepel leaf extract.

Table 4. Digestibility of Protein

Parameter	Treatment			
	T0	T1	T2	T3
Protein Digestibility (%)	48.63[a]	63.65[b]	67.03[c]	49.03[a]

Note: Different superscripts in the same column show very significant differences (P<0.01);
T0: basal ration; T1: basal ration + 0.15% Kepel leaf extract;
T2: basal ration + 0.30% Kepel leaf extract; T3: basal ration + 0.45% Kepel leaf extract.

Table 5. Feed Consumption and Body Weight Gain (BWG)

Parameter	Treatment			
	T0 _	T1	T2	T3
Feed Consumption (g/bird)	2031.76	2024,36	1828,18	1835,69
BWG (g/bird/d)	35,36	41,01	36,35	36,80

Description: T0: basal ration; T1: basal ration + 0.15% Kepel leaf extract; T2: basal ration + 0.30% Kepel leaf extract; T3: basal ration + 0.45% Kepel leaf extract.

CONCLUSION

The inclusion of 0.30% Kepel leaf extract in the feed significantly affects the number and width of the ileum villi. This improvement in villi condition was followed by an increase in protein digestibility in broiler chickens. However, the addition of Kepel leaf extract to the feed did not significantly affect broiler body weight gain, ration consumption, the number of *E. coli* bacteria count in excreta, and height of ileal villi.

REFERENCES

Amalia H. (2020). Pengaruh Pemberian Ekstrak Daun Burahol (*Stelechocarpus Burahol*) dalam Ransum terhadap Jumlah Bakteri *Enterobacteriaceae* dan Nilai Ph Digesta Ileum Ayam Broiler. Unpad *Repository*.

Amananti W, Tivani I, Riyanta AB. (2017). *Uji Kandungan Saponin pada Daun, Tangkai Daun dan Biji Tanaman Turi (Sesbania Grandiflora)*. 2nd Applied Science and Technology National Seminar (SENIT) 2017. Tegal: Indonesia

Awad, W.A., K. Ghareeb, S. Nitclu S. Pasteiner, S.A. Raheem, and J. Bohm. 2008. *Effect of dietary inclusion of probiotic, prebiotic, and symbiotic on intestinal glucose absorption of broiler chickens*. Lrt. J. Poult. Sci. 7: 688- 691.

Bambang, A.G., Fatimawali, S.N. Kojong. 2014. *Analisis Cemaran Bakteri Coliform dan Identifikasi Escherichia Coli Pada Air Isi Ulang Dari Depot di Kota Manado.Jurnal Ilmiah Farmasi*. Manado: Universitas Sam Ratulangi.

Barton, M.D. & W.S. Hart. 2001. *Public health risks: Antibiotic resistance*. Asian Australian Journal of Animal Science, 14: 414-422.

Biowey RW and Weaver AD. 2003. *Color Atlas of Diseases and Disorders of Cattle* 2nd Ed. USA (US): Elsevier Limited.

BPOM RI. 2011. Acuan Sediaan Herbal. 6(I). BPOM. Jakarta.

Brooks, G.F., S.B. Janet, A.M. Stephen, M. Jawetz, dan Adelbergs. 2008. *Medical microbiology II*. Salemba Medika. Jakarta

Djojoningrat. 2006. Buku Ajar Ilmu Penyakit Dalam: *Dispepsia Fungsional*. Jakarta: Penerbit Buku Kedokteran EGC.

Febriani, S. 2017. Kecernaan Protein dan Organ Imunitas Broiler Diberi Pakan Mengandung Kacang Koro Pedang (*Canavalia ensiformis*) Dengan Taraf Protein Berbeda dan Penambahan Enzim Protease. Departemen Ilmu Nutrisi dan Teknologi Pakan. IPB: Bogor.

Halimatunnisroh, R., T. Yudiarti dan Sugiharto. 2017. Jumlah Coliform, BAL dan Total Bakteri usus halus Ayam Broiler yang diberi kunyit (Curcuma domestica). J. Peternakan Indonesia. 19 (2): 79-84.

Hidayat, A. A. 2010. Metode Penelitian Kesehatan Paradigma Kuantitatif: *Health Books*, Surabaya.

Ibrahim, S. 2008. Hubungan ukuran-ukuran usus halus dengan berat badan broiler. Agripet: Vol (8) No. 2: 42-46.

James, R. G. 2004. *Modern Livestock and Poultry Production*. 7th ed. Thomson Delmar Learning Inc., FFA Activities, London

Japfa Comfeed Indonesia. 2012. Performa Broiler MB 202. PT. JCI. Jakarta.

Jayanegara A, Ridla M, Laconi EB, Nahrowi. 2019. Komponen Antinutrisi pada Pakan. Penerbit IPB Press. Bogor.

Lusandika EH., Suarjana IGK., Suada IK. 2017. Kualitas Air Peternakan Ayam Broiler Ditinjau dari Jumlah Bakteri Coliform dan *Escherichia coli*. Denpasar (ID): Universitas Udayana.

Mayer, R., Stecher, G., Wuerzner, R., Silva, R. C., et. al. 2008. *Proanthocyanidins: Target Compounds as Antibacterial Agents*. J. Agric. Food Chem., 56 (16).

Mountzouris, K. C., P. Tsitrikos, I. Palamadi, A. Arvaniti, M. Mohnl, G. Schatzmayr, and K. Fegeros. 2010. *Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition*. Poult. Sci. 89 (1): 58 – 67.

Nuryana, R. S. (2020). Potensi Daun Kepel sebagai *Feed Additive* dalam Ransum Terhadap Absorpsi Nitrogen dan Mortalitas Ayam Broiler. Tesis. Universitas Padjadjaran. Indonesia.

Pusat Laboratorium Forensik (Puslabfor) Polri. 2019. Analisis Zat Bioaktif Ekstrak Daun Kepel dengan Alat LC-MS. Puslabfor Polri: Jakarta.

Radji M.A, Adekeye J.O, Kwaga JKP and Bale JOO. 2003. *In Vitro and In Vivo Pathogenicity Study of Escherichia coli Isolat from Poultry in Nigeria*. 58: 1-6.

Ranjhan, S.K. 1980. *Animal Nutrition and Feeding Practice in India*. New Delhi. Vikan Pub. House P.U.T. Ltd.

Robinson, T., (Institut Teknologi Bandung). (1995). Kandungan Organik Tumbuhan Tinggi. *Chemistry Progress*. https://doi.org/10.1109/ TKDE.2010.80

Schneider, B.H. and Flatt, W.P. 1975. *The Evaluation of feeds through digestibility experiment*. The University of Georgia Press. Athens.

Sutrisno, V. D. Yunianto & N. Suthama. (2013). Kecernaan Protein Kasar dan Pertumbuhan Broiler yang Diberi Pakan Single Step Down dengan Penambahan Acidifier Asam Sitrat. *Animal Agriculture Journal*. 2(3): 48-60.

Wiryawan, K. G., S. Luvianti, W. Hermana, dan S. Suharti. (2007). Peningkatan performa ayam broiler dengan suplementasi daun salam [*Syzygium polyanthum (Wight) Walp*] sebagai Antibakteri *Escherichia coli*. Media Peternakan. 30 (1): 55-62.

Yamakoshi, J., S. Kataoka, H. Hosoyama, dan T. Ariga. 2002. *Deodorant agents for oral use for discharges and method for relieving odor of discharges*. US Patent, 6395280.