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COMBINATION EFFECT OF WATER EXTRACT HERBAL LEAVES ON DRINKING WATER TO STIMULATE THE GROWTH AND BIO-CONTROL OF PATHOGENIC BACTERIA IN BROILER

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ABSTRACT

This study aims to examine the effect of giving a combination of extract water from *Sauropus androgynus* (SL) and *Moringa oleifera* (ML) leaves through drinking water to stimulate the growth and health of broiler chickens. A total of 240 broiler chickens day-old chicks (DOC) with a homogeneous mean initial body weight of 40.19 ±0.18 g were randomly divided into four treatments and six replications in a completely randomized design. Drinking water treatments included: drinking water without herbal leaf extract as a control (A); drinking water with 3% ML water extract (B); drinking water with 3% SL water extract (C); and drinking water with 1.5% *ML* water extract+1.5% *SL* water extract (D), respectively. The results showed that the weight gain, feed efficiency, digestibility of feed in Group B, C, and D chickens were significantly (P<0.05) higher than Group A. In contrast, serum cholesterol levels and the number of *Choliform* and *E. choli* in chicken digesta decreased significantly (P<0.05). It can be concluded that the addition of *Moringa* leaf water extract, *Sauropus*, and their combination in broiler drinking water can improve broiler performance, reduce serum cholesterol levels and reduce the number of pathogenic bacteria.

Keywords: Phytochemicals, cholesterol, pathogenic bacteria, broilers

INTRODUCTION

The ban on the use of antibiotic growth promoters (AGPs) in poultry feed in Indonesia as of January 2018 has caused significant panic among medium-scale chicken farmers, because feed production and efficiency have dropped and chickens are very susceptible to disease. Therefore, several studies have tried to find alternatives to these AGPs by utilizing water extracts of herbal plants that are easily available around their cages. Compounds isolated from herbal leaves have anti-oxidant, anti-carcinogenic, anti-diabetic, anti-inflammatory, and anti-hypertensive properties (Ashok *et al.,* 2014; Dalukdeniya *et al*., 2016; Elangovan *et al*., 2014; Godinez-Oviedo *et al.,* 2016). However, the results showed different results. This is thought to be due to the dose of administration, the type of extract, and the method of administration in poultry (Chukwuebuka, 2015).

Plants that have the potential for medicine (herbal) are quite many types and have been used quite widely for medicinal purposes. It is very important to know the traditional properties of herbal plants, because they will increase the diversity of vegetable resources and form the basis of economic and other applied botanicals. Therefore, the use of herbal plants and spices is an alternative that can be used as a substitute for commercial feed supplements in rations, as a substitute for using AGPs (Bidura, 2020). Interesting to study its properties as a natural feed supplement is a combination of water extract from *Sauropus* leaves (*Sauropus androgynus*) and *Moringa oleifera* leaves. The phytochemical compounds contained in it are: saponins, flavonoids, and tannins (Oka *et al*., 2016; Putra *et al*., 2016; Santoso *et al*., 2015; Bidura *et al*., 2017; Ayssiwede *et al*., 2011; Chukwuebuka, 2015; Siti *et al*., 2019), and several other phenolic compounds that have antimicrobial activity (Sudatri *et al*., 2019; Bukar *et al*., 2010). Flavonoids that resemble estrogen are able to lower blood cholesterol levels and increase high density lipoprotein (HDL) levels, while saponins have been shown to have anticancer, antimicrobial, and lower blood cholesterol levels. As reported by Xiong *et al.,* (2012) and Zhu *et al.,* (2009) stated that flavonoids with estrogenic effects were shown to significantly improve the growth performance of chickens, improve carcass quality, eliminate free radicals, and increase body antioxidants, as well as increase immunity in certain additive ranges in poultry.

The combined effect of the two types of herbal extracts is expected to cause a synergistic effect of the two phytochemical compounds of the herbal leaves, so as to improve the health of chickens and the ability to digest feed, so that feed efficiency can be increased. Based on this, this study aims to examine the effect of giving a combination of extract water from *Sauropus androgynus* (SL) and *Moringa oleifera* (ML) leaves through drinking water to stimulate the growth and health of broiler chickens.

MATERIALS AND METHODS

Experimental design, animals, housing and diets

A total of 240 broiler chickens day-old chicks (DOC)

with a homogeneous mean initial body weight of 40.19 ±0.18 g were randomly divided into four treatments and six replications in a completely randomized design. Drinking water treatments included: drinking water without herbal leaf extract as a control (A); drinking water with 3% *Moringa oleifera* leaf (ML) water extract (B); drinking water with 3% *Sauropus androgynus* leaf (SL) water extract (C); and drinking water with 1.5% *ML* water extract+1.5% *SL* water extract (D), respectively. All rations given were the same, namely isocaloric and isonitrogenous rations (Table 1).

The rations (Table 1) were formulated to meet the nutritional requirements recommended by Scott *et al.,* (1982). The ration was given in the form of mash. All chickens were housed in environmentally controlled rooms with controlled ventilation and wire floors. Each cage was equipped with a nipple drinker and a plastic feed holder. Feed was given *ad libitum* to all chickens and all chickens were given free access to drinking water and feed during the trial period. The ingredients and chemical compositions of the feed are shown in Table 1.

Method of making water extract of Moringa leaves and *Sauropus* leaves.

Fresh *Moringa* leaves and *Sauropus* leaves were obtained from the research location. Each herbal leaf is weighed as much as 1 kg and put in 1 liter of clean water (v/v; 1: 1). Furthermore, the leaves are kneaded until crushed, then let stand for 24 hours (cold maceration) at room temperature. After 24 hours, the extract was then filtered and put into a 1 liter capacity plastic bottle. The herbal leaf water extract was ready for using in research, which was given through drinking water continuously during the trial period.

Performance

During the experiment, continuous lighting and access to feed and water were provided throughout the day. Birds are weighed at baseline (one day of age) as initial body weight and at 35 days, as final body weight. Feed intake was measured based on cage plots (10 birds/cage) every week. The daily feed intake per bird was calculated based on the total feed intake in one cage plot for the entire trial period and for the number of days in all periods. Feed conversion ratio (FCR) is the ratio between feed consumption and body weight gain (kg feed/kg live weight gain) for all treatments. Blood serum cholesterol levels were analyzed following the Liberman-Burchard method (Lieberman and Burchard, 1980).

Measurement of Feed Digestibility

The calculation of nutrient digestibility (dry matter and organic matter digestibility) was determined by using a total collection technique for three days, when the chicken was 4 weeks old. The amount of feed given was calculated every day as much as 100 g per head, as long as Likewise, the observation of the digesta collection was carried out for three days Before the experiment was carried out, all the chickens were fasted for 12 hours, which aims to ensure that the digestive tract was empty of leftover feed, but drinking water was still provided. Drinking water was available *ad libitum* during observation. Total excreta was collected in a plastic tray. The excreta samples were dried in the sun, then in an oven at 105^0C, weighed, and finely ground through a 1 mm filter. Furthermore, the excreta and ration samples were analyzed to determine the dry matter (DM) and organic matter (OM) content. The determination of dry matter (DM) and organic matter (OM) was carried out according to to the Assocciation of Official Analytical Chemists method (2005), and samples were analyzed in duplicate. To determine the dry matter digestibility coefficient (% DM)) and organic matter digestibility (% OM) the ration was based on dry matter (DM) with the formula:

$$\text{DM digestibility (\%)} = \frac{\text{nutrient consumption - faecal nutrient x 100\%}}{\text{Faecal nutrient}}$$

Table 1. The ingredient and calculated nutrient content of the feed of broiler aged 0-5 weeks

Basal Diets	**Compositions**	
Ingredients (%)*:*		
Yellow corn	46.5	
Rice bran	10	
Coconut meal	12	
Soybean meal	16	
Fish meal	15	
Mineral-B12*)	0.5	
Total	100	
Chemical composition **)*:*		
Metabolizable energy	(kcal/kg)	2922
Crude protein	%	22.5
Ether extract	%	7.56
Crude fibre	%	4.88
Calcium	%	1.48
Phosphor-availabel	%	0.79
Arginine	%	1.74
Histidine	%	0.55
Isoleusine	%	1.16
Leusine	%	1.99
Lysine	%	1.61
Methionine	%	0.5
Phenilalanine	%	1.08
Treonine	%	0.96
Tripthophan	%	0.26
Valin	%	1.18

) The mineral composition of B12 per 10 kg contains: Calsium: 49%; Phosphor 14%; Iron: 40000 mg; Manganese: 27500 mg; Mg: 27.500 mg; Zinccum: 25 mg; Vit-B12: 4.50 mg and Vit D3: 500000 IU. PT. Eka Farma. Deptan RI No. D 8109127 FTS

**) Based on calculation according to Scott *et al.,* (l982)

***Total plate count* (TPC)**

TPC is a technique for calculating the number of all microbes present in the digesta using Plate Count Agar (PCA) media. The analysis of total plate count digesta was in the following way, namely: 3 grams of digesta was put into an Erlenmeyer tube which already contains 90 ml of sterile 0.1% peptone water solution, so that a 10^{-1} dilution is obtained. This 10^{-1} dilution was homogenized and diluted again by taking 1 ml with a pipette, then put it in a test tube containing 9 ml of pepon solution, so that a 10^{-2} dilution is obtained. And so on, in order to obtain a dilution to 10^{-6}. Then, planting was carried out using the pouring method as practiced by Nuriyasa *et al.,* (2020). This planting was done in a sterile room and close to a bunsen fire. It aims to avoid contamination from the outside environment, by taking a dilution level of 10^{-5}; 10^{-6}; and 10^{-7} with a pipette. Then poured with PCA media at a temperature of ± 45^0C into a 20 ml petri dish and closed again. Then homogenized by moving the petri dish carefully, then leaving it until the media solidifies. Planting was made in duplicate (duplo) in an incubator with a temperature of 37^0C and reversed. The results can be calculated after 24-48 hours in the incubator.

Testing for *Coliform* and *Escherichia coli* bacteria:

The method used to obtain total *Escherichia coli* and *Coliform* bacteria was the spread method according to

Table 2. The effect of herbal leaf extract (ML, SL, and its combination) on drinking water on the number of *TPC, Choliform*, *Eschericia coli* (cfu g^{-1}) in digesta and feed digestibility of broiler

Variables	Treatments[1)]				SEM[2)]
	A	B	C	D	
TPC (cfu g^{-1})	$3.82x10^{7a3)}$	$4.03x10^{7a}$	$5.39x10^{7a}$	$5.94x10^{7a}$	2.371
Choliform (cfu g^{-1})	$8.05x10^{4a}$	$4.79x10^{4b}$	$3.06x10^{4b}$	$2.81x10^{4b}$	1.137
E. choli (cfu g^{-1})	$7.82x10^{3a}$	$3.65x10^{3b}$	$3.72x10^{3b}$	$2.18x10^{3b}$	1.045
Feed digestiblity					
• Dry matter (%)	70.05b	73.69a	73.41a	73.82a	1.084
• Organic matter (%)	71.72b	75.54a	75.49a	75.76a	1.107

Note:
1. Drinking water without herbal leaf extract as a control (A); drinking water with 3% ML water extract (B); drinking water with 3% SL water extract (C); and drinking water with 1.5% *ML* water extract + 1.5% *SL* water extract (D), respectively.
2. Standart error of the treatment means
3. Means with different superscripts within raw values are not significantly different (P>0.05)

Table 3. The effect of herbal leaf extract (ML, SL, and its combination) on drinking water on growth performance and blood lipid profile in broiler

Variables	Treatments[1)]				SEM[2)]
	A	B	C	D	
Initial body weight (g $head^{-1}$)	40.17a[3)]	39.97a	40.26a	40.09a	0.018
Final body weight (g $head^{-1}$)	1950.83b	2119.36a	2104.29a	2135.72a	45.036
LWGs (g 35 $days^{-1}$)	1910.66b	2079.39a	2064.03b	2095.63a	43.942
Feed consumption (g 35 $days^{-1}$)	3209.91a	3098.29a	3137.33a	3101.53a	40.894
FCR (FI:LWGs)	1.68a	1.49b	1.52b	1.48b	0.041
Blood lipid profile (mg dl^{-1})					
• Cholesterol total	157.08a	143.91b	139.62b	138.73b	4.052
• Triglycerides	107.51a	97.35a	99.24a	97.06a	2.075
• Hingh density lipoprotein (HDL)	84.63a	86.52a	86.98a	88.05a	3.724
• Low density lipoprotein (LDL)	22.35a	20.93a	21.78a	21.07a	0.807

Note:
1. Drinking water without herbal leaf extract as a control (A); drinking water with 3% ML water extract (B); drinking water with 3% SL water extract (C); and drinking water with 1.5% *ML* water extract + 1.5% *SL* water extract (D), respectively.
2. Standart error of the treatment means
3. Means with different superscripts within raw values are not significantly different (P>0.05)

Fardiaz (Sriyani *et al.,* 2020), which uses EMBA media, as much as 5 cc of intestinal digesta was inserted into the Erlenmeyer tube which already contains 0.1% peptone water solution with a volume of 45 ml, so a 10^{-1} dilution was obtained. This 10^{-1} dilution was then homogenized and diluted again by taking 1 ml through a pipette and then putting it into a test tube containing 9 ml of peptone solution, so that a 10^{-2} and 10^{-3} dilution was obtained. From 10^{-1} dilution, it was taken using a sterile pipette of 0.1 ml, then poured on the surface of the solid EMBA media into a petri dish then incubated at 37^0C in reverse, and the results can be calculated after 24-48 hours. Planting was carried out at dilution levels of 10^{-1}, 10^{-2}, and 10^{-3}. Counting the number of growing bacterial colonies using the plate count method, namely by selecting the number of colonies growing on a petri dish ranging from 30-300 colonies (Nuriyasa *et al.,* 2020) with the following formula:

$$\text{Colony/gram} = \text{Number of colonies per plate} \times \frac{1}{\text{Dilution factor}}$$

Statistic analysis

All data obtained were analyzed for one-way variance. For microbiological data, before being analyzed, it was first transformed into a logarithmic form. If the analysis results show a significant difference (P<0.05), then continue with Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The results showed that the addition of 3% water extract of *Moringa* leaves (ML), *Suropus* (SL), and the combination through chicken drinking water, did not have a significant effect (P>0.05) on the amount of TPC in chicken digesta. However, it significantly (P<0.05) decreased the number of *Choliform* and *E.choli* bacteria (Table 2). Administration of ML and SL extracts through drinking water during the study period significantly (P<0.05) decreased the number of *Choliform* and *E.choli* bacteria in the digestive tract of chickens (Table 2). Mohammed (2020) reported that the ethanol extract of plant leaves has the ability to be used as an antibiotic against infection-causing bacteria. Types of herbal extracts turned out to have different abilities to inhibit *Candida albicans* and *Trichophyton mentagrophytes* (Mohammed *et al*., 2017). The same thing was reported by Mohammed (2020) that the types of herbal extracts used turned out to have different inhibitory effectiveness against harmful bacteria.

Dry matter digestibility in Group B, C, and D chickens, were significantly (P<0.05) increased: 5.20%; 4.80%; and 5.38% higher than Group A chickens (Table 2). Likewise, the organic digestibility of Group B, C, and D chickens, were significantly increased (P<0.05) higher: 5.33%; 5.26%; and 5.63% higher than Group A (control). The results of the research by Olugbemi *et al.,* (2010) and Siti *et al.,* (2019) reported that supplementation of 2-6% *Moringa* leaf meal in the ration of laying hens significantly improved the digestibility of dry matter and organic matter of feed. It was also reported that the phytochemical compounds in *Moringa* leaves have the ability to suppress the number of *Choliform* and *E.choli* bacteria, so that absorption of nutrients can be optimal. Similar to the research of Carvalho *et al.,* (2019) who found that the addition of 2-4% herbal leaf water extract (*Tamarindus indica*) in the drinking water of laying hens can significantly improve the digestibility of dry matter and organic feed ingredients.

The effect of adding ML, SL water extracts, and their combinations to broiler drinking water on final body weight, live weight gain, feed intake (FI), FCR, and blood lipid profiles (total cholesterol, triglycerides, HDL, and LDL) were presented in Table 3. Feed intake, triglyceride levels, HDL, and LDL in the blood of Groups A, B, C, and D chickens were not significantly different (P>0.05). The average live weight gains (LWGs) in Group B, C, and D chickens was significantly different (P<0.05), namely: 8.83%; 8.03%; and 9.69% higher than chickens in Group A. Meanwhile, between chickens in Group B, C, and D, there were no significant difference (P>0.05). Wibawa *et al.,* (2016) found that the addition of Garlic's water extract in the drinking water of broiler chickens significantly increased the final body weight and live weight gains of chickens. The same thing was reported by Nuhu (2010) and Puspani *et al.,* (2019) that the use of herbal leaf flour (*Moringa*) and Carrots (*Daucus carota*) in rabbit rations, can significantly improve rabbit growth and feed efficiency.

The FCR value is an indicator to assess the level of feed use efficiency. The lower the FCR value, the higher the feed efficiency. Conversely, the higher the FCR value, the lower the feed efficiency. The average FCR value in group A chickens was 1.68. The average FCR values in Group B, C, and D chickens were: 11.31%; 9.52%; and 11.90% were significantly (P<0.05) lower than Group A, while the FCR values between Group B, C, and D chickens were not significantly different (P>0.05). The content of phytochemical compounds in ML, SL, and their combinations can help absorb nutrients. According to Xiong *et al.,* (2012) and Zhu *et al.,* (2009), flavonoid compounds with estrogenic effects were shown to significantly improve the growth performance of chickens, eliminate free radicals, and increase the body's antioxidants, as well as increase the immunity of poultry. The results of this study are the same as Wibawa *et al.,* (2016) who reported that adding Garlic extract to chicken drinking water significantly increased feed efficiency.

The results in Table 3 show the lipid profile of the serum, due to the administration of leaf water extract (ML, SL, and their combinations) on drinking water for broiler chickens. Triglyceride, HDL, and LDL levels

in chicken blood serum Groups A, B, C, and D, did not show any significant difference (P>0.05). The mean total cholesterol level in Group A chicken serum was 157.08 mg/dl. Cholesterol content in blood serum for Group B, C, and D chickens were: 8.38%; 11.12%; and 11.68% were significantly (P<0.05) lower than Group A (control) chickens. This shows that the herbal extract can significantly reduce the cholesterol content in the chicken body. As reported by Sudatri *et al.,* (2019); Dalukdeniya *et al.,* (2016); Elangovan *et al.,* (2014); Godinez-Oviedo *et al.,* (2016), that compounds isolated from herbal leaves have antioxidant, anticarcinogenic, and antihypertensive properties. Bidura *et al.,* (2017) stated that giving aqueous extract of *Sauropus* leaves 5 cc/100 cc of drinking water given to laying hens can significantly reduce the cholesterol content in egg yolks. Likewise, the research results of Wibawa *et al.,* (2016) found that the water extract of Garlic in drinking water significantly decreased serum cholesterol content in chickens. The dose of extract administration, the type of extract, and the method of administration to poultry can affect the response to the results obtained (Chukwuebuka, 2015).

CONCLUSION

It is concluded that the addition of water extract *Moringa oleifera*, *Sauropus androgynus* leaf and their combination in broiler drinking water can improve broiler performance, reduce serum cholesterol levels and reduce the number of pathogenic bacteria in digesta of broiler.

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REFERENCES

AOAC. (2005). Official Methods of Analysis of AOAC International. 18th Ed. Association of Official Analytical Chemists, Arlington, USA.

Ashok, V., Gomashe, P.A.G., Megha, P.J., Neeta, A.D. (2014). Antimicrobial Activity of Indian Medicinal Plants: *Moringa oleifera* and *Saraca indica. Int. J. Curr. Microbiol. App. Sci*., 3(6): 161-169.

Ayssiwede, S.B., A. Dieng, H. Bello, C.A.A.M. Chrysostome, M.B. Hane1, A. Mankor, M. Dahouda, M.R. Houinato, J.L. Hornick, and A. Missohou (2011). Effects of *Moringa oleifera* (Lam.) leaves meal incorporation in diets on growth performances, carcass characteristics and economics results of growing indigenous Senegal chickens. *Pakistan Journal of Nutrition* 10(12): 1132-1145

Bidura, I.G.N.G. (2020). Daun Herbal dan Probiotik Alternatif Pengganti Antibiotik Growth Promotors (AGPs) Pada Ternak. Penerbit Swasta Nulus, Jl. Tukad Batang hari VIB No. 9, Denpasar-Bali

Bidura, I.G.N.G., I.B.G. Partama, B.R.T. Putri, and N.L.Watiniasih (2017). The effect of water extract of two leaves (*Allium sativum* and *Sauropus androgynus*) on the egg production and yolk cholesterol level in layer hens. *Pakistan Journal of Nutrition*, 15(1): 23-31

Bukar, A., T.I. Uba, and Oyeyi. 2010. Antimicrobical profile of *Moringa oleifera* Lam. ekstracts against some food-borne microorganism. *Bayero Journal of Pure and Applied Sciences*, 3(1): 43-48.

Carvalho, M.D.C.D., I.B.G. Partama, I.G.N.G. Bidura and N.W. Siti (2019). Effect of drinking water supplementation with extract fermented *Tamarindus indica* by *Saccharomyces spp* on egg production, feed digestibility and N-NH3 content in excreta laying hens. *International Journal of Multidisciplinary Approach and Studies*, 6(5): 19-28

Chukwuebuka, E. (2015). *Moringa oleifera* "The Mother's Best Friend". *International Journal of Nutrition and Food Sciences,* 4(6): 624-630

Dalukdeniya, D.A.C.K., K.L.S.R. De Silva and R.M.U.S.K. Rathnayaka (2016). Antimicrobial activity of different extracts of leaves bark and roots of *Moringa oleifera* (Lam). *Int. J. Curr. Microbiol. App. Sci.* 5(7): 687-691.

Elangovan, M., Dhanarajan, M.S., Rajalakshmi, A., Jayachitra, A., Mathi, P., Bhogireddy N. (2014). Analysis of phytochemicals, antibacterial and antioxidant activities of *Moringa oleifera* Lam. leaf extract- an *in vitro* study. *Int. J. Drug Dev. & Res*., 6(4): 173-180.

Godinez-Oviedo, A., N. Guemes-Vera and O.A. Acevedo-Sandoval (2016). Nutritional and Phytochemical composition of *Moringa oleifera* Lam and its potential use as nutraceutical plant: A Review. *Pakistan Journal of Nutrition*, 15(4): 397-405

Lieberman, A. and R. Burchard (1980). Enzimatic method to determined cholesterol. *Engl. J. Med.*, 271: 915-924.

Mohammed, H.A. (2020). Effect of antibacterial activity of some plant extracts on opportunistic bacteria. *Plant Archives,* 20 (2):. 4815-4821

Mohammed, H.A., M.E. Al-Defiery and N.K. Imran (2017). The efficiency of some plant extracts for growth inhibition of *Candida albicans* and *Trichophyton mentagrophytes. Baghdad Sci. J.,* 14(2): 262-269.

Nuhu, F. (2010). Effect of *Moringa* leaf meal (MOLM) on nutrient digestibility, growth, carcass and blood indices of weaner rabbits. Msc. Thesis, Animal Science Department of the Faculty of Agriculture and Natural Resources/Kwame Nkrumah University of Science and Technology: Kumasi, pp: 107.

Nuriyasa, I.M., I.K. Sukada, E. Puspani And, N.T. Ariana (2020). Microbial Composition Of Hind Gut,

Digestability And Growth Rate Of Local Rabbit With Feed Fermentated Banana Peels (Acuminata Balbisiana) Supplementation. *Plant Archives*, 20(2): 6334-6338

Oka, A.A., K.A. Wiyana, I.M. Sugitha dan I.N.S. Miwada (2016). Identification of functional Properties of teak leaves, *Moring*a and *Cinnamon* and its potential as a source of antioxidants in edible film. *Jurnal Sain Peternakan Indonesia,* 11(1): 1-7

Olugbemi, T.S., S.K. Mutayoba and F.P. Lekule (2010). Effect of Moringa (Moringa oleifera) inclusion in cassava based diets fed to broiler chickens. *Int. J. Poult. Sci*., 9: 363-367.

Puspani, E., I.G.N.G. Bidura, I. K. Sumadi, I.M. Nuriyasa and D.P.M.A. Candrawati (2019). Growth Performance, Meat Cholesterol and β-Carotene Content in Rabbit Fed With Carrot Leaves, Grass, and Concentrates. *J. Biol. Chem. Research* 36(2): 27-33

Putra, I.W.D.P., A.A.G.O. Dharmayudha, L.M. Sudimartini (2016). Identification of chemical compounds ethanol extract leaf *Moringa* (*Moringa Oleifera*) in Bali. *Indonesia Medicus Veterinus*, 5(5): 464-473

Santoso, U., Y. Fenita, Kususiyah and I.G.N.G. Bidura (2015). Effect of Fermented *Sauropus androgynus* leaves on, meat composition,. Amino Acid and Fatty acid compositions in broiler chickens. *Pakistan journal of Nutrition*, 14(11): 759-807

Scott, M. L., M. C. Neisheim, M.C. and R. J. Young. (l982). Nutrition of The Chickens. 2nd Ed. Publishing by: M. L. Scott and Assoc. Ithaca, New York.

Siti, N.W., I.G.N.G. Bidura, S.N. Mayuni and I.A. Utami (2019). Effect of Moringa oleifera Leaf Powder in Diets on Feed Digestibility and External Egg Quality Characteristics in Laying Hens. *J. Biol. Chem. Research*, 36(2): 80-87

Sriyani, N.L.P., N.L.G. Sumardani and I.G. Suarta (2020). Effect of carcass aging length on physical and microbiological quality of balinese pork. *Plant Archives*, 20(2): 7261-7265

Sudatri, N.W., N. Wirasiti, N.M. Suartini and I.G.N.G. Bidura (2019). Anti-diabetic and anti-cholesterol activity of *Kaempferia galanga* L. herbal medicine rhizome in albino rats. *International Journal of Fauna and Biological Studies*, 6(5): 13-17

Wibawa, A.A.P.P., I.A.P. Utami and I.G.N.G. Bidura (2016). The effect of Garlic (*Allium sativum*) extract on performance, abdominal fat and serum cholesterol of broiler. Journal of Biological and Chemical Research, 33(1): 214-225

Xiong, XW., PF. Zhou and JH. Ding (2012). Effect of alfalfa meal and alfalfa flavonoids excrement on growth performance and nutrient utilization of Chongren chicken female. *Jiangxi J. Anim. Husb. Vet. Med.,* 3: 30-33.

Zhu, JM., N. Li, and YJ. Zhang (2009). Advances in alfalfa flavonoids. *Acta Pratacult Sin.* 26: 156-62.