World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

IDENTIFICATION OF PHYTOCHEMICAL COMPOUNDS IN MORINDA CITRIFOLIA AND IMPLEMENTATION OF MORINDA LEAF JUICE VIA DRINKING WATER ON CARCASS AND ABDOMINAL FAT IN MALE BALI DUCKS (ANAS SP.)

Ni Wayan Siti*, A. A. P. P. Wibawa and I. G. N. G. Bidura

Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia.

Corresponding Author: Ni Wayan Siti
Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia.

Article Received on 20/08/2021 **Article Revised on 10/09/2021** **Article Accepted on 30/09/2021**

ABSTRACT

The purpose of this study was to identify the content of phytochemical compounds in *Morinda citrifolia* leaves, and to test *Morinda* leaf juice (MLJ) through drinking water to increase carcass weight and reduce abdominal fat in male Bali ducks (*Anas sp.*). The design used in this feeding trial was a completely randomized design with four treatments and six replications, in which each experimental unit used 10 male Bali ducks aged two weeks with homogeneous body weight, so that the total ducks used were 4x6x10 birds = 240 birds. The four treatments were: ducks that were given drinking water without using MLJ (B); ducks given drinking water contained 2% MLJ (C); and ducks given drinking water contained 3% MLJ (D). All rations given were commercial rations for ducks with 18% protein content and 2900 kcal/kg metabolized energy. Ration and drinking water are provided ad libitum. The results showed that the phytochemical compounds contained in *Morinda citrifolia* leaves include: tannins, flavonoids and triterfenoids which are strongly positive, while the content of saponins is relatively weak. Implementation of MLJ in drinking water at a concentration of 1-3%, significantly ($P<0.05$) can increase feed consumption, carcass weight and carcass percentage in ducks. Abdominal fat of ducks decreased significantly ($P<0.05$) in the group of ducks given the water extract of Noni leaves. It can be concluded that MLJ supplementation at a concentration of 1-3 cc/100 cc in drinking water of ducks from 2-8 weeks of age can increase feed consumption and carcass weight, and decreased abdominal fat.

KEYWORDS: *Flavonoid, tannin, carcass, abdominal fat, duck.*

INTRODUCTION

Morinda citrifolia plant is a tree with a height of 4-8 m. Stem woody, round, leathery, and branching monopoidal. Single leaf, ovate, pointed tip and base, and about 10-40 cm long. Compound interest, hump shape, stemmed, stamens 5. Fruit hump, irregular surface, fleshy, 5-10 cm long, yellowish green (Syamsul Hidayat and Hutapea, 1991). *Morinda* fruit contains scopoletin, rutin, polysaccharides, ascorbic acid, β-carotene, 1-arginine, proxironin, and proxeroninase, iridoids, asperolusid, iridoid anthraquinone, fatty acids, calcium, B vitamins, amino acids, glycosides, and glucose (Sjabana and Bahalwan, 2002).

In large-scale poultry rearing systems, there are often obstacles to being attacked by pathogenic bacteria. The bacteria that often attack the digestive tract of poultry is *Escherichia coli* which causes colibacillosis in broilers, so that broiler productivity decreases during the rearing period (Wahyuwardani et al., 2014). Breeders generally use antibiotics to treat these diseases, but currently the use of antibiotics is prohibited because excessive use of antibiotics is feared to cause allergies in consumers, due to antibiotic residues in meat, disturbances in the balance of microorganisms in the digestive tract, and resistance of microorganisms to antibiotics (Mutmainah et al., 2014). Therefore, it is necessary to make efforts to replace the use of antibiotics by utilizing the efficacy of herbal leaves. According to Bidura (2020), alpha flavonoids with estrogenic effects have been shown to significantly improve growth performance, improve carcass quality, eliminate free radicals, and increase body antioxidants, as well as increase body immunity within a certain additive range in livestock.

Herbal leaves are generally high in beta-carotene content, and the absorption of beta-carotene by the body will increase with increasing fat intake, because beta-carotene is fat-soluble. Beta-carotene is widely used as a feed supplement, because it is one of the antioxidants that has the benefit of preventing various diseases, increasing endurance, and providing analgesic and anti-inflammatory effects (Bidura, 2020). The properties of beta-carotene which are insoluble and easily oxidized in

water is a problem in the formulation of beta-carotene for oral application related to its absorption and bioavailability in the body (Dixon et al., 2013).

The decrease in cholesterol and triglyceride content by alkaloids is partly due to reduced activity of lipogenic enzymes and increased excretion of bile acids in feces. The presence of phytochemical compounds, such as flavonoids, saponins, tannins, and flavonoids, are able to reduce blood cholesterol levels and increase HDL levels, while saponins have been shown to be efficacious as anticancer, antimicrobial, and reduce cholesterol in serum and yolk (Santoso *et al.,* 2015; Bidura *et al.,* 2017; Bidura et al., 2021). Hestera (2008) reported that the use of phytochemical compounds from *Moringa oleifera* leaves and *Curcumin* leaves in feed can reduce the cholesterol content of chicken meat. Utilization of plant herbal properties is an alternative that can be used as a substitute for commercial feed supplements in drinking water, such as antibiotics (Bidura, 2020).

Interesting to study its efficacy as a natural feed supplement is *Morinda citrifolia* leaf juice. *Morinda citrifolia* plant is one source of supplements that have good potential. This *Morinda* plant is a medicinal plant with potential to be developed. Almost all parts of the *Morinda* plant contain various substances that are useful for treatment and maintaining a healthy body. *Morinda* leaves contain protein, lime, iron, carotene, and ascorbin. Research Setyaningsih (2011) reported that as much as 2.5-10% *Morinda* flour given to the boiler can reduce cholesterol levels in the breast meat of boiler. Mutmaimah et al. (2014) and Mulyani (2014) reported that the parts of herbal plants that are usually used as medicine are the rhizome and the leaves. Administration of herbal leaf extracts (*Moringa* and *Allium*) as much as 50 ml/liter via drinking water given to broilers significantly reduces abdominal fat and cholesterol levels in broiler serum (Restiayanti et al., 2014) and yolk cholesterol (Bidura et al., 2017).

Based on this, researchers are interested in identifying the content of phytochemical compounds in *Morinda citrifolia* leaves and the effect of giving *Morinda* leaf juice in ducks seen from the carcass and abdominal fat aspects.

MATERIALS AND METHODS

Feeding trial and experimental design. Sample analysis was carried out at the Integrated Service Laboratory, Faculty of Agricultural Technology, Udayana University; and the GC-MS test was carried out at the Integrated Laboratory, FMIPA Udayana University, Denpasar. The design used in this feeding trial was a completely randomized design with four treatments and six replications, in which each experimental unit used 10 male Bali ducks aged two weeks with homogeneous body weight, so that the total ducks used were 4x6x10 heads = 240 heads. The four treatments were: ducks that were given drinking water without using MLJ (B); ducks given drinking water contained 2% MLJ (C); and ducks given drinking water contained 3% MLJ (D). All rations given were commercial rations for ducks with 18% protein content and 2900 kcal/kg metabolizable energy. Ration and drinking water were provided *ad libitum*.

Measurement of feed and drinking water consumption variables was measured once a week, namely the difference between the amount of feed/water given and the remaining feed/drinking water. Final body weight was measured at the end of the study. Before weighing, the ducks were fasted for 12 hours. Slaughter of ducks, begins with cutting the artery in the neck (*Altlanto occipitalis*) to remove blood from the body of the duck. After the ducks die, the next step was to remove the feathers. To facilitate the removal of feathers, the dead ducks were immersed in hot water at a temperature of 70^0-80.2^0C for 0.5-1.0 minutes. After that, the duck body parts were separated, namely the expulsion of the digestive tract, internal organs, cutting of the legs and head, and finally the carcass was obtained. Removal of internal organs and digestive tract was done surgically in the abdomen, except for the crop organ which was removed by dissecting the layer of skin at the base of the ventral neck that covers the crop. Separation of the head and neck was done by cutting the *Altlanto occipitalis* joint, which was the junction between the atlas bone (*Cervical vertebrae*) and the skull. To separate the legs was done by cutting the joint *Tibio tarsometatarsus.* Pad-fat is fat on the abdominal pads and abdominal fat is fat in the abdominal cavity including: mesenteric fat, ventriculus fat, and pad fat.

The process of making *Morinda* leaf juice

First, *Morinda citrifolia* leaves were prepared from the *Morinda citrifolia* fruit plantation in Penebel District, Tabanan Regency, Bali Province, Indonesia. *Morinda* leaves used leaves that are old to young (not shoots), and were still green. *Morinda* leaves were then washed and cut into small pieces, after that 1 kg of *Morinda* leaves were blended and mixed with 1 liter of water. *Morinda* leaf juice was then filtered and ready to be given to ducks through drinking water according to the treatment (1-3 cc/100 cc of drinking water). The process of making *Morinda* leaf juice was presented in Figure 1.

Fig. 1: The process of making Morinda leaf juice before giving it to ducks via drinking water.

The ethanol extract of *Morinda* leaves and phytochemical screening were carried out at the Integrated Service Laboratory, Faculty of Agricultural Technology, Udayana University and the GC-MS test was carried out at the Integrated Laboratory, FMIPA, Udayana University, Denpasar. The main ingredient of this research was *Morinda* leaf water extract. After the extraction of these materials, phytochemical tests and GC-MS tests were carried out, then in vitro tests of the two extracts were carried out on *E. coli* bacteria to see their inhibition against the growth of *E. coli* bacteria.

Production of *Morinda* Leaf extract and phytochemical tests

Morinda leaf extract was made from 1 kg of *Morinda* leaves, washed with water until clean, then finely chopped and air-dried in a room without direct sunlight, until the weight was constant and becomes simplicia. Furthermore, the simplicia was blended until smooth, sieved, and macerated in 96.5% ethanol and filtered, so that the filtrate was obtained. The remaining filtered dregs were macerated again with the same process, the filtrate obtained was combined with the first filtrate. Then the filtrate was evaporated with a rotary evaporator until a more concentrated solution was formed, so that a crude extract was obtained and was ready to be used in the next test (Kurniawan et al., 2014). Phytochemical screening was carried out through a series of tests using certain reagents. This phytochemical screening was carried out in accordance with that carried out by Fitriani and Nugraheni (2015). Phenol and flavonoids can be detected using 1% $FeCl_3$ solution in ethanol. The test result was considered positive if it produces green, red, purple, blue or black colors. The shinode test (concentrated Mg and HCl) can also be used to detect flavonoids. Flavonoids will show a very strong cherry red color when sprayed with this reagent. After the phytochemical screening was carried out, the *Morinda* leaf extract was then tested with the GC-MS test to identify the types of active compounds in these ingredients.

Statistical Analysis

The data obtained were analyzed in one-way ANOVA and if there was a significant difference ($P<0.05$) between treatments, it was continued with Duncan's multiple-distance test.

RESULT

After the phytochemical screening was carried out, the *Morinda citrofilia* leaf extract was then tested with the GC-MS test to identify the types of active compounds in these ingredients. The results of laboratory analysis to identify the content of phytochemical compounds in Noni (*Morinda citropilia* L.) leaves are presented in Table 1. Noni leaves contain strong positive tannins, flavonoids and triterfenoids, while the saponins content is relatively weak. In contrast, alkaloids and phenol compounds were not detected or were negative. The content of beta-carotene is quite high, which is 15230 mg/100 leaves.

Table 1: The content of phytochemical compounds in *Morinda citropilia* L. leaves.

The group of phytochemical compounds in *Morinda citropilia L.* leaves.							
Alkaloid	**Fenol**	**Tanin**	**Saponin**	**Flavonoid**	**Steroid**	**Triterpenoid**	**β-caroten (mg/100 g)**
-	-	+++	+	+++	++	+++	15230

Note: – = Negative, + = weak positive, ++ = positive, dan +++ = strong positive

Table 2 presents data on final body weight, feed and drinking water consumption, carcass weight, carcass percentage (carcass weight/slaughter weight x 100%), pad fat, and abdominal fat. The administration of MLJ at a concentration of 1-3% in drinking water significantly

increased (P<0.05) final body weight, slaughter weight, feed and drinking water consumption in ducks.

The average carcass weight and carcass percentage of ducks in the duck group that received MLJ supplementation via drinking water increased significantly (P<0.05) compared to control (without MLJ supplementation). On the other hand, there was a significant decrease (P<0.05) in abdominal fat, while the pad-fat showed no significant difference (P>0.05). More details are presented in Table 2.

Table 2: Characteristics of carcass and abdominal fat in male Bali ducks (*Anas sp.*) aged 8 weeks given MLJ via drinking water.

Variables	MLJ concentration in drinking water (cc/100 cc)				SEM[1]
	0	1	2	3	
Feed consumption (g/days)	73.47b[2]	78.88a	81.34a	79.89a	1.036
Drinking water (cc/days)	215.49b	252.93a	267.38a	259.05a	10.827
Final body weight (g)	1108.57b	1238.19a	1275.14a	1242.05a	25.379
Carcass weight (g)	623.71b	746.11a	758.49a	744.88a	32.913
Carcas percentages (%)	56.19b	60.17a	59.35a	59.83a	1.035
Pad-fat (% body weight)	0.38a	0.40a	0.37a	0.36a	0.081
Abdominal-fat (% body weight)	0.73a	0.62b	0.64b	0.60b	0.026

Note:
1. Standart error of the treatment means
2. Means with different superscripts within row values are significantly different (P<0.05)

DISCUSSION

The results showed that the administration of MLJ through drinking water could increase feed consumption, as well as duck body weight. This increase was due to the MLJ containing phytochemical compounds, such as saponins, flavonoids, and tannins (Table 1) and several other phenolic compounds that have antimicrobial activity, such as saponins which have been shown to have antimicrobial properties (Bukar et al., 2010). The decrease in the number of pathogenic bacteria in the intestine provides opportunities for optimal absorption of nutrients in the intestine, so that body weight and carcass can be maximized. Restiayanti et al. (2014) reported that supplementation of herbal extracts in broilers did not affect feed consumption, but could significantly increase body weight gain. Likewise, the results of research by Alçiçek et al. (2003, 2004) that supplementation of herbal mixtures into broiler feed, significantly increased live weight and feed efficiency. In contrast, the research of Hammershøj et al (2010) reported that feed consumption decreased significantly in chickens that were given herbal supplements compared to chickens that were not given herbal supplements. Reported by Erhan et al. (2012) that the Herbal *Pennyroyal* supplement reduces feed consumption and feed efficiency in broilers. William and Rosa (2001) reported that the observed increase in chicken weight gain was due to the opening properties of plant extracts by increasing gastric digestive juices and establishing a more balanced intestinal flora with their antimicrobial effect. As reported by Ürüşan et al. (2018), that cold-pressed carrot seed oil supplementation can cause an increase in slaughter weight, carcass weight, and broiler carcass percentage. The same thing was reported by Alçiçek et al. (2003; 2004) that supplementation of herbal mixtures into broiler feed, significantly increased live weight and carcass characteristics of broilers.

Morinda leaf juice supplementation through drinking water at a level of 1-3% significantly increased the weight gain and carcass of ducks. This increase in body weight is closely related to increased protein consumption as a result of increased feed consumption, as well as the presence of phytochemical compounds in Noni leaves. Tang et al. (2007) stated that the increased consumption of protein and amino acid lysine in broilers caused an increase in breast meat compared to lower consumption of protein and lysine. Feeds that contain high protein will increase the components of meat in the body. The same thing was reported by Al-Batshan and Hussein (1999) that increasing protein consumption would increase carcass weight, carcass percentage, and breast meat. According to Babic et al. (1994) and Yu et al. (2005), the increase in body weight can be attributed to the antioxidant and antimicrobial properties contained in herbal leaves. Contrary to the research results of Prabowo et al. (2019) which stated that the administration of herbal leaf flour (*Carrot* leaves) into the ration actually reduced the protein mass of the meat, while the calcium mass of the meat had no significant effect. Feed efficiency in the duck group that received MLJ was significantly better than the control group (without MLJ). As with other herbal leaves, Noni leaves contain high levels of beta-carotene (Table 1). Beta-carotene or pro vitamin A in Noni leaves is converted into vitamin A which plays a role in the differentiation of epithelial cells and maintains the digestive organs, so that it will affect the digestibility and efficiency of feed. Increased feed efficiency and feed digestibility in broilers can affect protein synthesis and calcium intake which can affect the level of protein mass and calcium mass in meat (Prabowo et al., 2019). The Calislar study (2019) has reported that beta-carotene compounds can boost the immune system by increasing antibody

response in poultry and preventing acute respiratory infections.

Microbial balance in the digestive tract occurs when the composition consists of 85% beneficial microbes and 15% pathogenic microbes (Sjofjan, 2003). The decrease in the number of harmful bacteria in the digestive tract of ducks will result in optimal absorption of nutrients, which will have an impact on improving the performance of ducks. Several researchers reported that the use of herbal leaves in rations could reduce the number of pathogenic bacteria. Several researchers reported that supplementation of *Turmeric* powder (Ürüşan and Bölükbaşı, 2017), *Pennyroyal* (Erhan et al., 2012), and orange peel oil (Erhan and Bölükbaşı, 2017) in broiler diets could increase the number of lactic acid bacteria and decrease *Escherichia coli* bacteria in the jejunum. On the other hand, Steenfeldt et al. (2007) reported that the number of *Choliform* bacteria and lactic acid bacteria in the small intestine was not affected by the addition of carrots to the diet of laying hens.

Abdominal fat in the group ducks that received 1-3% MLJ supplementation in drinking water decreased significantly compared to the control duck group (without MLJ supplementation). Noni leaves contain tannins, saponins, flavonoids, steroids, and triterpenoids (Table 1). Giving 50 cc/100 cc of herbal leaf extract to broilers significantly reduced abdominal fat and serum cholesterol levels (Restiayanti et al., 2014) and yolk cholesterol in hens (Bidura et al., 2017). Contrary to the results of Ürüşan et al. (2018) research, that the addition of Carrot seed oil supplementation to the basal ration had no effect on serum biochemical parameters. It was also reported that carrot seed oil supplementation resulted in positive changes in body weight gain, carcass yield, breast meat, and the number of lactic acid bacteria in the intestine. Berliana et al. (2020) reported that supplementation of black garlic flour in the ration had no effect on protein mass and fat mass in broiler breast meat. Carrot leaf herb showed the effect of reducing cholesterol absorption in experimental rats (Gramenzi and Gentile, 1990).

CONCLUSION

Phytochemical compounds contained in *Morinda citrofolia* leaves are: tannins, flavonoids, triterfenoids, saponins and beta-carotene as much as 15230 mg/100 g. *Morinda citrifolia* leaf juice supplementation at the level of 1-3 cc/100 drinking water can increase carcass and reduce abdominal fat in male Bali ducks (Anas sp).

AKNOWLEDGEMENTS

Thank you to the Dean of the Faculty of Animal Science, Udayana University for the Study Program research grant with a contract Number: B/34/UN14.2.3 /PT.01.05/2021, so that the research up to the writing of this article can be completed on time. Thanks are also extended to the Laboratory analysts who have assisted in sample analysis.

REFERENCES

1. Al-Batshan, H.A. and E.O.S. Hussein. Performance and carcass composition of broiler under heat stress: The effect of dietary energy and protein. Asian-Austalian Journal Animal Science, 1999; 2(6): 914-922.
2. Alçiçek, A., M. Bozkurt, and M. Çabuk. The effects of an essential oil combination derived from selected herbs growing wildin Turkey on broiler performance. S. Afr. J. Anim. Sci., 2003; 33(2): 89-94.
3. Alçiçek, A., M. Bozkurt, and M. Çabuk . The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. S. Afr. J. Anim. Sci., 2004; 34(4): 217-222.
4. Babic, I., C. Nguyen-the, M.J. Amiot, and S. Aubert. Antimicrobial activity of shredded carrot extracts on food-borne bacteria and yeast. J. Appl. Bacteriol., 1994; 76: 135-141.
5. Çalişlar, S. 2019. The important of beta-carotene on poultry nutrition. Selcuk J Agr Food Sci., 2019; 33(3): 256-263. http://sjafs.selcuk.edu.tr/sjafs/index.
6. Erhan, M.K., and Ş.C. Bölükbaşı. Citrus peel oils supplementation in broiler diet: effects on performance, jejunum microflora and jejunum morphology. Rev. Bras. Cienc. Avic, 2017. Special Issue Nutrition/015-022. http://dx.doi.org/10.1590/1806-9061-2016-0274.
7. Erhan, M.K., Ş.C. Bölükbaşi, and H. Ürüşan. Biological activities of pennyroyal (*Mentha pulegium* L.) in broilers. Livest Sci., 2012; 146: 189-192.
8. Berliana, Nelwida dan Nurhayati. Breast meat mass protein and fat of broiler chicken consumed diet containing black Garlic. Sains Peternakan, 2020; 18(1): 15-22. DOI: http://dx.doi.org/10.20961/sainspet.v%vi%i.32174.
9. Bidura, I.G.N.G. Daun Herbal dan Probiotik Alternatif Pengganti Growth Promotors (AGPs) pada Ternak. Penerbit Swasta Nulus. Jl. Batanghari, Denpasar-Bali, 2020.
10. Bidura, I.G.N.G., I.B.G. Partama, B.R.T. Putri, and N.L.Watiniasih. 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, 2017; 15(1): 23-31.
11. Bidura, I.G.N.B., N.W. Siti, A.A.P.P. Wibawa, I.N. Tirta Ariana and E. Puspani. The effect of Carot leaves meal fermented in diets on egg production, yolk cholesterol and beta-carotene in yolk of hens. Annals of the Romanian Society for Cell Biology, 2021; 25(6): 18705-18711. https://www.annalsofrscb.ro /index.php/journal/article /view/9400/6856.
12. Bukar, A., T. I. Uba and Oyeyi. Antimicrobical profile of *Moringa oleifera* Lam. ekstracts against

some food-borne microorganism. Bayero Journal of Pure and Applied Sciences, 2010; 3(1): 43-48.
13. Dixon, J.L., Kim, Y.K., Brinker, A. and Quadro, L. Loss of beta-carotene 15,15′-oxygenase in developing mouse tissues alters esterification of retinol, cholesterol and diacylglycerols. Biochim. Biophys. Acta, 2013; 1841: 34-43.
14. Fitriani, A.F. and Nugraheni F.D. Analaisis organik daun kunyit. *Makalah Kimia.* Kementrian Perindustrian RI Pusat Pendidikan dan Pelatihan Industri Politeknik AKA Bogor, 2015; 1-32.
15. Gramenzi, A. and Gentile, A. Association between certain foods and risk of acute myocardial infarction in women. BMJ, 1990; 300: 771-773.
16. Hestera, T. S. Efek Penggunaan Tepung Daun Kelor dalam Pakan Terhadap Persentase Karkas Persentase Deposisi Daging Dada Persentase Lemak Abdominal dan Kolesterol Daging Ayam Pedaging. Program Studi Nutrisi dan Makanan Ternak, Fakultas Peternakan, Universitas brawijaya, Malang, 2008.
17. Hammershoj M., Kidmose U. and S. Steenfeldt. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (Daucus carota) varieties as forage material for egg-laying hens. Journal of the Science of Food and Agriculture, 2010; 90: 1163–1171.
18. Kurniawan, R. W. A. Y., Wiratmin, N. I., and Sudatri, N. W. Histologi hati mencit (*Mus Musculus* L.) yang diberi ekstrak daun lamtoro (*Leucaena leucocephala*). *Simbiosis*, 2014; 2(2): 226–235.
19. Mulyani, S., B.A. Harsojuwono, G.A.K.D. Puspawati. Potensi minuman kunyit asam (*Curcuma domestica* val. -*Tamarindus indica* L.) sebagai minuman kaya antioksidan. *Agritech*, 2014; 34(1): 65–71.
20. Mutmainah, R.S., N.Rahmawati[c], Nugroho, A.E. Gastroprotective effects of combination of hot water extracts of turmeric (*Curcuma domestica* L.), cardamom pods (*Ammomum compactum* S.) and sembung leaf (*Blumea balsamifera* DC.) against aspirin–induced gastric ulcer model in rats. Asian Pacific Journal of Tropical Biomedicine, 2014; 4(1): 500-504.
21. Prabowo, L. D. Mahfudz, dan U. Atmomarsono. Calcium and protein meat mass due to the use of waste product of Carrot powder in ration. Jurnal Sain Peternakan Indonesia, 2019; 14(2): 201-207. DOI: https://doi.org/10.31186/jspi.id.14.2.201-207.
22. Restiayanti, L., I.G.N.G. Bidura dan N.L.G. Sumardani. Pengaruh pemberian ekstrak daun Kelor (*Moringa oleifera Lam*) dan daun bawang putih (*Allium sativum*) melalui air minum terhadap distribusi lemak tubuh dan kadar kolesterol broiler umur 2-6 minggu. E-jurnal Peternakan Tropika, 2014; 2(3): 402.
23. Santoso, U., Y. Fenita, Kususiyah and IGNG. Bidura. Effect of fermented *Sauropus androgynus* leaves on, meat composition, amino acid and fatty acid compositions in broiler chickens. Pakistan journal o nutrition, 2015; 14(11): 759 – 807.
24. Setyaningsih, E. Pengaruh penambahan tepung mengkudu (*Morinda citrifolia* L) dalam ransum terhadap penurunan kadar kolesterol daging ayam broiler strain Hubbard. Prosiding Seminar Biologi., UNS, Solo. Halaman, 2011; 145-152.
25. Sjabana, D. dan Bahalwan, R.R. Seri Referensi Herbal: pesona Tradisional dan Ilmiah Buah mengkudu (*Morinda citrifolia*, L). Salemba Medika, Jakarta. 2002; https://ccrc.farmasi.ugm.ac.id/?page_id=389.
26. Sjofjan, O. Kajian probiotik (*Aspergillus niger* dan *Bacillus spp*) sebagai imbuhan ransum dan implikasi efeknya terhadap mikroflora usus serta penampilan produksi ayam Petelur. Disertasi. Universitas Padjajaran, Bandung, 2003.
27. Steenfeldt, S., J.B. Kjaer, and R.M. Engberg. Effect of feeding silages or carrots as supplements to laying hens on production performance, nutrient digestibility, gut structure, gut microflora and feather pecking behaviour. Br. Poult. Sci., 2007; 48: 454-468.
28. Syamsul Hidayat, S.S dan Hutapea, J.R. Inventaris Tanaman Obat Indonesia.edisi kedua. Departemen Kesehatan RI. Jakarta, 1991. https://ccrc.farmasi.ugm.ac.id/?page_id=389.
29. Tang, M. Y., Q. G. Ma, X. D. Chen and C. Ji. Effects of dietary metabolizable energy and lysine on carcass characteristics and meat quality in Arbor acres broiler. AJAS, 2007; 20(12): 1865-1873.
30. Ürüşan, H., M. K. Erhan and S. C. Bölükbaşı. Effect of cold-press Carrot seed oil on the performance, carcass characteristics, and shelf life of broiler chickens. The Journal of Animal & Plant Sciences, 2018; 28(6): 1662-1668.
31. Ürüşan H., and Ş.C. Bölükbaşı. Effects of dietary supplementation levels of turmeric powder (*Curcuma longa*) on performance, carcass characterıstıcs and gut mıcroflora in broiler chickens. J. Anim. Plants Sci., 2017; 27(3): 732-736.
32. Wahyuwardani, SS., Noor S M, M. Poeloengan, T. Aryanti. Cases of colibacillosis in chickens farm in Yogyakarta and Bogor. Seminar Nasional Teknologi Peternakan dan Veteriner, 2014. 606–10.
33. Williams, P. and R. Losa. The use of essential oils and their compounds in poultry nutrition. World Poultry-Elsevier, 2001; 17: 14-15.
34. Yu L.L., K.K. Zhou, and J. Parry. Antioxidant properties of coldpressed black caraway, carrot, cranberry, and hemp seeds oils. Food Chem, 2005; 91: 723-729.