World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Effect of fermented *Garcinia Mangostana* peel aqueous extract by *Effective Microorganism*-4 on growth performance and intestinal histology of broilers

Andika Firman [1], Ni Wayan Siti [2,*] and I Gusti Nyoman Gde Bidura [2]

[1] *Animal Science study program Magister, Faculty of Animal Husbandry, Udayana University, Denpasar, Indonesia.*
[2] *Faculty of Animal Husbandry, Udayana University, Denpasar, Indonesia.*

World Journal of Biology Pharmacy and Health Sciences, 2025, 21(02), 208-214

Publication history: Received on 01 January 2025; revised on 07 February 2025; accepted on 10 February 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.21.2.0160

Abstract

Interest in using herbal products as a source of antioxidants has increased in recent years, due to concerns about food safety and health. This study aims to determine the growth performance and intestinal histology of broilers given fermented aqueous extract of mangosteen peel (*Garcinia mangostana* L.) through drinking water. This research used a Completely Randomized Design (CRD) consisting of 4 treatments and 5 replications, each replication consisting of 8 broilers. The treatments given were: drinking water without fermented mangosteen peel aqueous extract (FMAE) as a control (P0), drinking water given 1.5% FMAE (P1); drinking water given 3% FMAE (P2), and drinking water containing 4.5% FMAE (P3). The results showed that broilers given aqueous extract of fermented mangosteen peel at levels of 1.5%, 3% and 4.5% (P1, P2 and P3) had no significant effect (P>0.05) on final weight, gain body weight, drinking water consumption, ration consumption, and Feed Conversion Ratio (FCR = feed consumption: body weight gain). Likewise, villi height, villi width and crypt depth showed no significant differences (P>0.05). Based on the research results, it can be concluded that the productivity of broilers given water extract of *Garcinia mangostana* peel fermented using *Effective Microorganism*-4 in drinking water has not been able to increase broiler productivity and improve intestinal histology. A more in-depth study is needed regarding the types of extracts and microorganisms used in fermentation.

Keywords: Mangosteen peel; Fermentation; Aqueous extract; Intestinal histology; Broiler

1. Introduction

Broiler farming is one of the main commodities which has a very important role in meeting the animal protein needs of society. The high consumption of broiler meat in Indonesia is due to its high nutritional value compared to other livestock. This cannot be separated from the various advantages that broilers have compared to native chickens, including a relatively short production period of approximately 32-35 days before they can be marketed, high productivity, relatively cheap prices and demand which is increasing every year [1].

To achieve maximum growth, broilers need good quality feed and environmental conditions. Good quality feed will be utilized optimally by livestock if the condition of the digestive tract is also good, because this is where the process of digestion and absorption of food substances takes place [2]. There are several beneficial bacteria such as lactic acid bacteria and also harmful bacteria (pathogenic bacteria), namely *Salmonella* bacteria, *Coliform* bacteria and *Eschericia coli* [3]. To control the growth of pathogenic bacteria, it is often done by adding antibiotic growth promoters (AGP) to the feed. However, this can be dangerous because it causes residue in the product and if consumed by humans it can cause antibiotic resistance [4].

*Corresponding author: Ni Wayan Siti

Broiler production businesses often use feed additives to prevent disease and increase growth, but it is not realized that continuous mixing of feed additives can cause the presence of metabolic products in the form of antibiotic residues, such as tylosin, penicillin, oxytetracycline and kanamycin [5]. One effort to overcome this problem is by providing herbal supplements that can replace the function of antibiotics and are not harmful to the body, namely aqueous extract of mangosteen peel (*Garcinia mangostana* L.) by mixing it with drinking water.

Mangosteen peel is one of the wastes that can be used as animal feed. Various research results regarding the use of mangosteen peel as an antioxidant, include [6] who states that the most important component of all mangosteen fruit is the peel, which is 70-75%, while the flesh is 10-15%, and the seeds are 15-20%. Mangosteen peel contains antioxidants, namely xanthones which have anti-cancer, antibacterial, antifungal and antiviral functions [7].

Interest in using herbal products as a source of antioxidants has increased in recent years, due to concerns about food safety and health, as well as the desire to improve the antioxidant quality and stability of the oxidative properties of meat [8]. The nutritional content of mangosteen skin is 62.05% water, 1.01% ash, 0.63% fat, 1.17% total sugar, 0.71% protein and 35.61% carbohydrates [9]. The research results of [10] reported that the use of mangosteen rind flour in rations did not have a significant effect on carcass percentage and percentage of broiler carcass parts. On the other hand, Candra [11] reported that giving 120 mg/kg body weight of mangosteen extract per day was able to increase chicken body weight gain and feed efficiency.

It was also reported by [12] that the use of mangosteen peel flour at a level of 3% in feed can increase carcass weight and broiler carcass percentage. Flavonoid compounds in herbal plants in low concentrations have the ability to inhibit the growth of pathogenic bacteria, so that the absorption of substances in food becomes more complete and the broiler's digestive tract can function optimally [13]. According to [14,15], flavonoid compounds in herbal leaves have an estrogenic effect, which can stimulate growth and carcass quality, eliminate free radicals, and increase the body's antioxidants and immunity. Reported by [16,17] that supplementation of 1-3% herbal leaf water extract in drinking water significantly increases carcass weight gain and feed efficiency. On the contrary, it significantly reduces the amount of abdominal fat and the population of pathogenic bacteria in the intestines of ducks and broilers.

The fermentation technique can be used to improve the quality of mangosteen peel, because it is simpler and can be done on small farms. *Effective Microorganism*-4 (EM-4) can be used as a fermentation inoculum, because it is reported to be a probiotic agent. Users will have an impact on the microbial ecosystem in the chicken's intestines, so that it can influence the population of pathogenic bacteria and increase feed digestibility, thereby increasing the efficiency of feed use and the health of the host animal [18]. In addition, fermented products are tastier than the original ingredients, because fermentation can increase feed palatability and water-soluble vitamins [19,20,21].

The use of probiotics as a feed ingredient can increase body weight, feed conversion and health in livestock and is a safe alternative ingredient because it promotes the growth of beneficial microbes and prevents the growth of pathogenic bacteria in the digestive tract. The aim of using probiotics is to increase growth, improve food digestion and increase the growth of beneficial microbes [18,22].

It is interesting to study the impact of giving water extract of *Garcinia mangostana* peel fermented with *Effective Microorganism*-4 (EM-4) on growth performance and intestinal histology of broilers.

2. Material and methods

2.1. Material

This research was carried out at Sesetan Farm, Faculty of Animal Husbandry, Udayana University, Jalan Raya Sesetan, Gg. Markisa Number 5, Sesetan, South Denpasar District, Denpasar City, Bali, Indonesia. The research material used was 160 day-old-chicken (DOC) broilers with homogeneous body weight and male sex. Broilers were obtained from PT. Poultry which is located at Jalan WR. Supratman, Number 281, Kesiman Kertalangu, Denpasar, Indonesia. The chicken coop used in this research used 20 "battery colony" cages with cage dimensions, namely length x width x height, namely: 100x60x75cm^3 made from bamboo strips, wood and wire mesh. Each cage plot was equipped with a feeder, drinking water container, lights and cage curtains to maintain the temperature of the cage. The base of the cage was filled with rice husks so that chicken droppings do not fall under the floor, making chicken droppings easy to clean and collect.

2.2. Effective Microorganism-4 (EM-4)

This research uses *Effective Microorganism* 4 (EM4) as a fermentation ingredient for aqueous extract of mangosteen peel obtained from PT. Tohpati Poultry which is located at Jalan WR Supratman, Number 281, Kesiman Kertalangu, Denpasar, Indonesia. Providing rations and drinking water was carried out *ad libitum* by paying attention to the availability of rations and drinking water in the feed container. The ration given in this study was the CP 511 Bravo ration which was given during the starter to finisher maintenance phase. The rations are produced by PT. Charoen Phokphand Indonesia, Tbk., Indonesia

2.3. Mangosteen Peel (*GarciniaMangostana* L.)

This research used mangosteen peel and used it as an extract for drinking water in the broiler rearing process. Mangosteen peel was obtained from the Badung market located in Denpasar City Center on Jalan Gajah Mada, Denpasar-Bali, Indonesia. Phytochemical content in aqueous extract of fermented mangosteen peel (*Garcinia mangostana* L.), were: flavonoid 25.80 mg/100g and tanin 23.16 mg/100g.

2.4. Experimental design

This research used a Completely Randomized Design (CRD) consisting of 4 treatments and 5 replications, each replication consisting of 8 broilers. The treatments given were: drinking water without fermented mangosteen peel aqueous extract (FMAE) as a control (P0), drinking water given 1.5% FMAE (P1); drinking water given 3% FMAE (P2), and drinking water containing 4.5% FMAE (P3), respectively

2.5. Measurement of research variables

This research looked at broiler performance variables which included: initial weight, feed consumption, drinking water consumption, final body weight, live weight gain, and feed conversion (Feed Conversion Ratio= feed consumption : live weight gain). Data tabulation was carried out every week during the research period.

Intestinal histology preparations were made by cutting the rumen of the small intestine 4 μm thick using a microton and placing them on slides for staining using the Hematoxylin-eosin method [23]. Then the finished villi preparations were taken to the laboratory to measure the number of villi, villi height, crypt depth and villi area. Observation of the number of villi was carried out by counting the number of villi in the histopath sample preparation using an Olympus BX51TF DIC microscope equipped with an Olympus DP 12 projector set to 4 times magnification. This villi height measurement uses the Image Raster application which has been calibrated according to the magnification of the microscope [24]. Then the depth of the crypt was measured from the base (the lamina propria) to the base of the villi [25].

2.6. Data Analysis

Data analysis in this study used one-way ANOVA. If there is a significant difference between treatments ($P<0.05$), proceed with Duncan's multiple range test [26].

3. Results and discussion

The results of research for 4 weeks of administering aqueous extract of fermented mangosteen peel in drinking water on final weight, live weight gain, drinking water consumption, feed consumption, and Feed Conversion Ratio (FCR) are presented in Table 1. Results of research on final weight of broilers without FMAE in drinking water (P0), administration of 1.5% FMAE (P1), administration of 3% FMAE (P2), and administration of 4.5% FMAE (P3), did not show any significant difference significant ($P>0.05$). Treatment P2 is 1.73% higher than P0.

The average value of feed consumption for broilers for 4 weeks given water extract of fermented mangosteen peel showed statistical results that were not significantly different ($P>0.05$). Broilers in treatment P1 consumed the highest ration of 0.027%; 0.32%; and 0.58% compared to treatments P0, P2, and P3.

The results of research on the Feed Conversion Ratio (FCR) value in broilers during the 4 week research period in the P3 treatment obtained results of: 3.05%; 4.88%; and 0.61% lower compared to treatments P0, P1, and P2, but statistically not significantly different ($P>0.05$).

Table 1 Effect of giving fermented aqueous extract of mangosteen peel (*Garcinia mangostana* L.) to broiler drinking water from 0-4 weeks of age on broiler growth performance.

Variable	**FMAE inclusion level in drinking water (cc/100 cc)**				**SEM**
	0	1.5	3.0	4.5	
Initial body weight, g	49.50	49.25	49.45	49.35	0.05
Final body weight, g	1340.00	1315.60	1363.60	1355.15	16.30
Live weight gain, g/4 weeks	1290.50	1266.35	1314.15	1305.80	16.29
Feed consumption, g/4 weeks	2252.55	2253.15	2245.95	2240.00	69.27
Drinking water, ml/4 weeks	5631.38	5632.88	5614.88	5600.01	76.93
Feed Conversion Ratio (feed consumption : live weight gain)	1.75	1.78	1.71	1.72	0.015

Note: Standard Error of Treatment Means

The administration of aqueous extract of fermented mangosteen peel to broiler drinking water from 0-4 weeks of age on intestinal histology including villi height, villi width and crypt depth is presented in Table 2.

Treatment P0 had an average height of intestinal villi of: 12.49%; 8.45%; and 15.73% significantly (P<0.05) higher compared to treatments P1, P2, and P3. Treatment P2 had a villi width of: 3.17%; 7.27%; and 24.47% higher compared to P0, P1, and P3, statistically not significantly different (P>0.05). Broilers in the P0 treatment had a crypt depth of: 16.64%; 32.80%; and 8.87% higher compared to P1, P2, and P3, statistically not significantly different (P>0.05).

Table 2 Effect of giving fermented aqueous extract of mangosteen peel (*Garcinia mangostana* L.) to broiler drinking water from 0-4 weeks of age on broiler intestinal histology.

Variable	**FMAE inclusion level in drinking water (cc/100 cc)**				**SEM**
	0	1.5	3.0	4.5	
Villi height (µm)	685.91^{a}	600.22^{b}	627.98^{b}	578.01^{b}	0.05
Villi width (µm)	154.95	148.39	160.02	120.86	4.99
Crypt depth (µm)	154.99	129.21	104.15	141.24^{a}	5.43

Note: The different superscript [^{a,b}] within the same row are significantlly different (p<0.05); SEM= Standard Error of the Treament Means

The inclusion of FMAE in the drinking water of broilers from 0-4 weeks of age did not have a significant effect on final body weight and weight gain of broilers. However, when administering 3% FMAE, the highest results were obtained. Mangosteen rind contains antioxidants, flavonoids, saponins and tannins which can increase the body's immunity against broilers, so that it can suppress pathogenic bacteria and help the digestive tract properly. Differences in final body weight and weight gain that are not significantly different are also influenced by feed consumption, especially energy and protein intake, which are almost the same, so the influence of feed consumption that is not significantly different can also influence final weight. The greater the amount of ration consumed, the greater the effect on increasing body weight and feed conversion ratio. This is in accordance with the statement of [27] who stated that the antioxidant compounds contained in the skin of the mangosteen fruit have a very important role in preventing damage caused by free radicals.

Increased use of rations can be influenced by lactic acid bacteria which play a role in increasing nutrient absorption. In addition, providing drinking water processed through fermentation can improve the microflora in the broiler's digestive system [28]. This is supported by [29] who said that feed consumption can be influenced by several factors, including; feed quality, growth rate, environmental temperature and health of livestock.

Providing FMAE up to a level of 4.5% reduces the FCR value or increases feed efficiency in broilers. The phytochemical content contained in the skin of the mangosteen fruit, including antioxidants, can increase nutrient absorption in the

broiler's body. This is caused by the administration of fermented water extract of mangosteen rind at a level of 1.5%; 3.0%; and 4.5% contains antioxidants, flavonoids, tannins and saponins, which is able to increase the broiler's immune system, so that it can suppress pathogenic bacteria and help the digestive tract properly. This shows that the water extract of mangosteen rind not only functions as an antioxidant, but also plays a role in increasing body weight and reducing the feed conversion ratio. In line with [30] statement which states that the smaller the FCR value, the more efficient the broiler will use feed, and vice versa. As is known, several studies have reported that the flavonoid content contained in mangosteen peel can increase immunity in livestock [31,32].

Broilers in treatment P0 had the highest average value of villi height and were statistically significantly different from treatments P1, P2, and P3. The high number of villi in P0 broilers is thought to be because P0 was not given FMAE, whereas treatments P1, P2, and P3 were given FMAE. Mangosteen peel contains organic compounds in the form of xanthones and is widely used to determine blood urea levels, fight cancer, control diabetes, reduce oxidation of low-density lipoprotein (LDL) in the blood, and reduce tissue damage due to free radicals [33]. The fermentation process with EM-4, which is a decomposing bacteria, means this compound is better digested by the stomach, so it does not reach the small intestine and does not have too significant an effect. Factors that influence villus height are the growth of villus height which is closely related to nutrient absorption and the length of the small intestine [34]. The length of the small intestine is influenced by the crude fiber content in the feed, the form of the feed, and the performance of the small intestine in digesting the heavier the feed causes the length of the small intestine to increase and conversely, the lighter the performance of the small intestine, the length of the small intestine does not increase [35]. According to [36] the higher the development of intestinal villi, the higher the absorption of nutrients in chickens, thus having an impact on the growth of other organs.

The treatment of fermented mangosteen water extract (P2) obtained the highest mean value of villi width of 160.02μm but was not statistically different. Providing aqueous extract of fermented mangosteen peel through drinking water will be directly absorbed by the digestive organs, so it has no significant effect on increasing the width of the broiler's intestinal villi. Increasing the width of the villi can provide a place for nutrient absorption and improve the performance of the small intestine [37]. According to [38] the increase in villi width is influenced by the age of the chicken, the older the chicken, the wider the small intestinal villi.

The increase in small intestine crypt depth is influenced by the presence of pathogenic bacteria in the small intestine. The deeper the crypt depth of the small intestine, the more pathogenic bacteria there are and this can lead to less nutrient absorption and more secretory cells [39]. According to [40] apart from pathogenic bacteria, crypt depth is influenced by the enzymes xylanse and phytase.

4. Conclusion

It can be concluded that the productivity of broilers given water extract of *Garcinia mangostana* peel fermented using *Effective Microorganism*-4 in drinking water has not been able to increase broiler productivity and improve intestinal histology. A more in-depth study is needed regarding the types of extracts and microorganisms used in fermentation.

Compliance with ethical standards

Acknowledgments

We would like to thank the Head of the Research Laboratory, Faculty of Animal Husbandry, Udayana University, Denpasar and the Head of the Integrated Services Laboratory, Faculty of Agricultural Technology, Udayana University, Denpasar for the research house and laboratory facilities.

Disclosure of conflict of interest

We authors declare that there is no conflict of interest with anyone.

Statement of ethical approval

The chickens and cages used in the experiments in this study were approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia.

References

[1] Ratnasari R, Sarengat W dan Setiadi A. Analysis of the income of broiler chicken farmers in a partnership system in Gunungpati District, Semarang, Indonesia. Animal Agriculture Journal, 2015; 4 (1): 47-53.

[2] Sukmawati NMS, Witariadi NM, Ardika IN and Siti NW. The effect of providing Mackerel herbs in the diet on the performance and number of pathogenic microbes in the intestines of broiler chickens. Jurnal Internasional Studi Fauna dan Biologi, 2022;9(6): 21-25.

[3] Halimatunnisroh R, Yudiarti T, Sugiharto. Total number of Coliforms, LAB and total small intestinal bacteria of broilers fed turmeric (*Curcuma domestica*). Jurnal Peternakan Indonesia. 2017;19(2): 81-87.

[4] Anggitasari S, Sjofan O dandjunaid IH. The influence of several types of commercial feed on the quantitative production performance of broiler chickens. Buletin Peternakan, 2016; 40(3): 196-287.

[5] Rusiana and Iswarawanti. Effect of antibiotics on broiler chicken meat and liver, Jakarta, Indonesia, 2004

[6] Iswari K. 2011. Highly Efficacious Mangosteen Peel. Centradifa Intermediate Publisher, Jakarta.

[7] Dondy. 2012. Utilization of Mangosteen Peel and Flour Technology. Bogor Agricultural Post-Harvest Research and Development Center. Warta Penelitian dan Pengembangan Pertanian 34(1): 12-13.

[8] Jiang J. dan Xiong YL. Natural Antioxidants as Food and Feed Additives to Improve the Health Benefits and Quality of Meat Products: A Review. Ilmu Daging, 2016; 120: 107-117

[9] Permana AW. 2011. Mangosteen Peel can be an instant drink rich in antioxidants. http://pustaka.litbang.deptan.go.i d/publikasi/wr322102 .pdf. [5 Desember 2013].

[10] Jebrizal. Muslim., dan Roza L, D. 2021. Effect of Giving Mangosteen Peel Extract (*GarciniaMangostana* L.) in Drinking Water on Broiler Carcass Percentage and Carcass Parts. Jounal of Animal Center. 3(1):1-10.

[11] Candra AA. Comparison of the Activity of Mangosteen Peel Water Extract and Various Antioxidants on Broiler Appearance. Jurnal Penelitian Pertanian Terapan, 2014; 15(1):68-74.

[12] Fitriani. 2023. Addition of Mangosteen peel flour (*Garcinia mangostana* L.) at different levels to the weight and percentage of broiler carcasses. Jurnal Gallus-Gallus, 2 (1):15-24.

[13] Robinson, T. 1995. High Plant Organic Content. ITB Publisher. Bandung, Indonesia

[14] Xiong, XW., PF. Zhou and JH. Ding. Effect of alfalfa meal and alfalfa flavonoids excrement on growth Performace and nutrient utilization of Chongren chicken female. Jiangxi J. Anim. Husb. Vet. Med., 2012; 3:30-33.

[15] Zhu JM, Li N, and Zhang YJ. Advances in alfalfa flavonoids. Acta Pratacult Sin. 2009; 26:156-62.

[16] Siti NW, Wibawa AAPP and Bidura IGNG. 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.). WJPLS, 2021;7(10): 167-172.

[17] Siti NW, Partama IBG, Wibawa AAPP, and Bidura IGNG. Combination effect of water extract herbal leaves on drinking water to stimulate the growth and bio-control of pathogenic bacteria in broiler. Plant Archives, 2021; 21(1): 1644-1649.

[18] Zurmiati, M. E. Mahata, M. H. Abbas, Wizna. 2014. The application of probiotic on duck. JurnalPeternakan Indonesia 16 (2): 134-144.

[19] Bidura IGNG, Partama IBG, Putri BRT, and Watiniasih NL. The ffect 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.

[20] Bidura IGNG, Siti NW, Candrawati DPMA, Puspani E and Partama IBG. Effect of probiotic *Saccharomyces* spp. On duck egg quality characteristics and mineral and cholesterol concentrations in eggshells and yolks. Pakistan Journal of Nutrition, 2019; 18 (11): 1075-1083 (https://scialert.net/abstract/?doi=pjn.2019.1075.1083

[21] Bidura, IGNG. Supplementation of *Saccharomyces* spp. probiotics into feed laying hens to increase feed digestibility and reduce ammonia gas content in excreta. Majalah Ilmiah Peternakan, 2020; 23(2): 84-90,

[22] Fuller R. Probiotic 2. Aplication and practical aspects. 1st. Ed. Chapman and Hall, London. 1997

[23] Emma WMSM, Sjofjan O, Widodo E, and Achmanu. 2013. Characteristics of the small intestine of broiler chickens given lime acid in feed. Jurnal Veteriner, 14 (1), 105-110.

[24] Harimurti S and Rahayu ES. Intestinal morphology of broiler chickens supplemented with single and mixed strain probiotics. Agritech, 2009; 29 (3): 179-183.

[25] Fard, S. H., Toghyani, M., and S. A. Tabeidion. 2014. Effect of Oyster mushroom wastes on performace, immune responses and intestinal morphology of broiler chickens. Int J Recycl Waste Agricult, 3 (1), 141-146.

[26] Steel RGD, Torrie JH. Principles and Procedures of Statistics. Translator BambangSumantri. Gramedia Pustaka; Jakarta, Indonesia: 2015.

[27] Kusumasari DP, Mangisah I and Estiningdriati I. The effect of adding vitamins A and E to the diet on egg weight and embryo mortality in black Kedu chickens. Animal agriculture journal, 2013; 2 (1): 191-200.

[28] Widodo W. Poisonous Plants in Livestock Life. Publisher Muhammadiyah University of Malang, Indonesia. 2002

[29] Rasyaf M. Guide to Raising Broiler Chickens. Jakarta: Self-Help Business Publishers, Jakarta, Indonesia. 2012

[30] Sumadi IK. Pig Nutrition 1th Ed. Publisher, Swasta Nulus, Denpasar; 2018.

[31] Knarreborg A, Simon MA, Engberg RM, Jensen BB, and Tannock GW. Effects of Dietary Fat Source and Subtherapeutic Levels of Antibiotic on The Bacterial Community in The Ileum of Broiler Chickens at Various Ages. Applied and Environmental Microbiology, 2002; 5918-5924.

[32] Lu J, Idris U, Harmon B, Hofacre C, Maurer J and Lee. MD. Diversity and Succession of the Intestinal Bacterial Community of the Maturing Broiler Chicken. Applied and Environmental Microbiology, 2003: 6816-6824.

[33] Monajjemi M, Azizi V, Amini SH and Mollaamin F. Nano theoretical studies on evaluation of anticancer potential on mangosteen plant. African Journal of Agricultural Research, 2011; 6(19): 4661-4670.

[34] Satimah S, Yunianto VD, and F. Wahyono F. Relative weight and length of the small intestine of broiler chickens fed a diet using microparticle egg shells supplemented with the probiotic Lactobacillus sp. JurnalSainPeternakan, 2019; 14(4): 396-403.

[35] Mustofa H, Guntoro EJ, and Supriyono. The effect of replacing some commercial rations with Indigoferasp leaf meal on the internal organs of broiler chickens (Gallus domesticus). Stock Peternakan, 2020; 2(2): 56-77.

[36] Ananda S, Hafizah A, Kiramang K, Jamili MA, Mutmainna A, and Rismawati. 2023. Profile of internal organs of broilers with the addition of probiotic *Effective Microorganism*-4 (EM-4) in drinking water. Journal Livestok and Animal Health, 2023; 6(1): 21-27.

[37] Budiartawan IKA, Darmawan IGAC, Berata IK, and Setiasih NLE. Histological development of duodenal villi of broiler chickens given butyric acid supplementation in feed. Indonesia Medicus Veterinus, 2018; 7(5): 522-530.

[38] Dwijayanti B, Rahmi E, Balqis U, Fitriani U, Masyitha D, Aliza D, and Akmal M. Histology, histomorphometry, and histochemistry of the intestine of free-range chickens (Gallus gallusdomesticus) during the period before and after hatching. Jurnal Agripet, 2021; 21(2): 128-140.

[39] Sirat MMP, Santosa PE, Ernawati R, Aini N, Lidyana A, and Rahma SW. Histomorphometry of broiler small intestine with supplementation of vitamin E, selenium and zinc in drinking water. Wahana Peternakan, 2023; 7(3): 281-293.

[40] Budiansyah A, Haroen U, Resmi, Syafwan, and Ramlah. Performance of broiler chickens treated with buffalo rumen fluid as a source of enzymes in corn and soybean meal based diets. Buletin Peternakan Tropis, 2023; 4(1): 69-87.