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Effect of water extract of *Mangosteen* peel (*Gracinia Mangostana* L.) fermented by probiotics on meat chemistry, abdominal fat and blood lipid profile of broilers

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Abstract

This study aims to determine the effect of administering fermented *Mangosteen* peel water extract (FMWE) in drinking water on meat chemistry, abdominal fat and blood lipid profile of broilers. The design used was a Completely Randomized Design (CRD), with 4 treatments with 5 replications and each replication using 40 day-old-chick (DOC) broilers with homogeneous body weight, so that a total of 160 DOC broilers were used. The treatments were: drinking water without FMWEas control (P0); drinking water+1.5% FMWE (P1); drinking water+3% FMWE (P2); and drinking water+4.5% FMWE (P3).The results of the study showed that giving 4.5% FMWE in broiler drinking water from 0-4 weeks of age had a significant effect (P<0.05) on reducing meat water content and meat ash content. On the other hand, it significantly (P<0.05) increased the fat content of broiler meat. The inclusion of 1.5-4.5% FMWE in broiler drinking water from 0-4 weeks of age did not have a significant effect (P>0.05) on cholesterol, low density lipoprotein (LDL) and abdominal fat levels. However, it significantly (P<0.05) increased the content of high-density lipoprotein (HDL) in broiler blood serum. It can be concluded that the administration of 4.5% water extract of fermented *Mangosteen* peel in broiler drinking water significantly reduces the water content and ash content of meat. On the contrary, it significantly increased the meat fat content and blood serum HDL levels of broilers.

Keywords: Abdominal fat; Blood lipid profile; Fermentation; Mangosteen peel aqueous extract

1. Introduction

Broiler meat is one of the livestock products that contributes to meeting the needs of animal protein sources for society. This couses broiler meat has a tender meat texture, is relatively cheap and contains protein which is good for growth, so it is liked by the public [1]. However, the rapid growth of broilers also produces meat that is fatty and high in cholesterol, namely containing 76 mg/100 g of cholesterol [2], so that consuming large amounts of broiler meat can cause atherosclerosis which has an impact on coronary heart disease [3].

The provision of feed additives in the form of Antibiotic Growth Promoter (AGP) in Indonesia has been prohibited since the issuance of regulation of the Minister of Agriculture of the Republic of Indonesia Number 14/Permentan/PK.350/5/2017 article 16 [4]. Because giving AGP creates residues in broiler meat which are dangerous for livestock and consumers by forming pathogenic bacteria.

Mangosteen fruit (*Garcinia mangostana* L.) is a phytochemical plant that is widely known in Indonesia. The mangosteen fruit has several parts, each of which consists of 70-75% *Mangosteen* peel, 10-15% fruit flesh and 15% fruit seeds [5]. *Mangosteen* peel is a waste that contains high levels of antioxidants found in xanthone compounds which are classified as polyphenols. The xanthones content in mangosteen peel extract is very high, namely 123.97mg/100ml [6]. Xanthones

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function as antitumoral, anti-inflammatory, antiallergic, antibacterial, antifungal and antiviral [7]. Xanthones are also often used to measure blood urea levels, prevent cancer, control diabetes, reduce the oxidation of blood low density lipoprotein (LDL) and inhibit damage caused by free radicals [8].

According to [9], the results of phytochemical screening show that *Mangosteen* peel also contains several phytochemical compounds, namely alkaloids, saponins, tripernoids, tannins, phenolics and flavonoids. According to [10,11,12], flavonoid compounds in herbal leaves have estrogenic effects, which can stimulate growth and carcass quality, eliminate free radicals, and increase antioxidants and body immunity.

Mangosteen peel has been widely used as animal feed [13]. However, mangosteen rind contains anti-nutrients, such as tannins which can have a negative effect on livestock productivity. The processing technique used is fermentation using probiotic microbes, such as *Effective Microorganism-4* (EM-4). *Effective Microorganism-4* (EM-4) is a probiotic that contains 90% lactic acid bacteria (*Lactobacillus sp*.), photosynthetic bacteria (*Rhodopseudomonas* sp.) and yeast (*Saccharomyces* sp.) [14]. Fermentation is a process of enzyme action by microbes that is able to break down complex components into simpler ones so that they are easily digested by livestock [15].

The benefits of fermentation with probiotic microbes are to increase nutritional content, reduce crude fiber and minimize anti-nutrient content, as well as improve the taste and odor of feed. The addition of the probiotic *Lactobacillus* sp. can also increase the protein content of meat and reduce fat in broilers [16]. Herbal plants that are fermented using probiotic microbes provide better benefits compared to those that are not fermented. Probiotic microbes are expected to have a synergistic effect with phytochemical compounds of herbal plants (*Mangosteen* peel), so that they can improve broiler health and the ability to digest feed [12]. The inclusion of probiotics in feed significantly increases nutrient absorption and reduces cholesterol and ammonia gas content in laying hens and ducks [17,18].

In the research results of [19] reported that providing fermented *Moringa* leaves at a level of 2-6% in feed can increase growth and feed efficiency, and conversely can reduce abdominal fat and pathogenic bacteria in the intestines of ducks. Saponin compounds in herbal leaves (*Carrots*), the main mechanism of their effect on reducing cholesterol in the body is through the transfer of cholesterol molecules from bile salt micelles, causing the formation of cholesterol deposits that cannot pass through the intestinal mucus layer [20]. The addition of 2-6% herbal leaf water extract to the drinking water of laying hens significantly reduces the cholesterol content in egg yolk [21,22]. This difference is caused by the type of herbal leaves used, the type of chicken, and the period of time the herbal extract was administered [12].

Based on the description above, this research needs to be carried out to determine the effect of giving fermented *Mangosteen* peel water extract by probiotics in drinking water on the chemical properties of meat, abdominal fat and the blood lipid profile of broilers.

2. Material and methods

2.1. Material

This research lasted for 4 weeks located at the Sesetan Research Station, Faculty of Animal Husbandry, Udayana University, South Denpasar, Indonesia. Analysis of blood samples was carried out at the Microbiology Laboratory, Faculty of Animal Husbandry, Udayana University and analysis of chemical meat samples at the Integrated Services Laboratory, Faculty of Agricultural Technology, Udayana University, Denpasar. The chickens used were 160 day-old-chick (DOC) broilers of the CP 707 strain produced by PT. Charoen Pokphand, Indonesia.

The cages used in this research used 20 "battery colony" cages with cage dimensions length x width x height, namely: 150 cm x 100 cm x 75 cm 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 tools used were: analytical scales, sieves, trays, knives, cutting boards, spoons, basins, closed containers (buckets), thermometers, syringes, EDTA tubes, label paper, boxes and stationery.

The ration used in this research was the BR1 CP 511 ration which was given during the starter maintenance phase to the finisher phase. The rations are produced by PT. Charoen Pokphand Indonesia, Tbk is in crumble form with a metabolizable energy content of 3000 kcal/kg and 23% protein.

2.2. Mangosteen peel (*Garcinia mangostana* L.) Fermentation Process

This research used *Mangosteen* peel extract (*Garcinia mangostana* L.) fermented in drinking water. Making *Mangosteen* peel extract fermented with probiotic microbes. The first step was to wash the *Mangosteen* peel with water until clean and then cut it into small pieces. Then, the *Mangosteen* peel was put into a bucket and sprayed with microbes at 5% of the weight of the material. After all the ingredients were mixed well, cover the bucket and ferment for 5 days. Next, the fermented *Mangosteen* peel was mixed evenly with water in a ratio of 5:1 (5 liters of water: 1 kg of *Mangosteen* peel). Then it was filtered using a double satin cloth, then put into a closed bottle and stored in the refrigerator. The results of the phytochemical analysis of fermented *Mangosteen* peel water extract are presented in Table 1.

Table 1 Results of laboratory analysis of phytochemical compound content of fermented *Mangosteen* peel water extract

Parameter	FMWE
IC_{50} (ppm)	22623.7837
Antioxsidant (mg/L GAEAC)	26.7646
Flavonoid (mg/100 g)	25.8090
Tanin (mg/100 g)	23.1605
Saponin	Negatif (-)

2.3. Experimental design

The design used was a Completely Randomized Design (CRD), with 4 treatments with 5 replications and each replication using 40 day-old-chick (DOC) broilers with homogeneous body weight, so that a total of 160 DOC broilers were used. The treatments were: drinking water without fermented *Mangosteen* peel water extract as control (P0); drinking water+1.5% water extract of fermented *Mangosteen* peel (P1); drinking water+3% water extract of fermented *Mangosteen* peel (P2); and drinking water+4.5% water extract of fermented *Mangosteen* peel (P3).

2.4. Observed Variables

The variables observed in this research were: meat chemistry which includes water content using the thermogravimetric method, protein content based on measuring the nitrogen content in the sample (carried out using the digestion, distillation and titration stages); fat content using a temperature of 105° C for 30 minutes until constant weight; and ash content using a furnace at a temperature of 500° C until it becomes ash characterized by a grayish white color without any black spots, then cool in a desiccator and weigh the crucible and ash [23].

Abdominal fat percentage: obtained by dividing the combination of pad-fat, mesenteric-fat, and ventricular-fat by the slaughter weight then multiplying by 100%. Blood lipid profile: Blood sampling was carried out at the end of the experiment and blood was taken through the auricular vein with an EDTA capillary tube. To obtain plasma, the blood was centrifuged at a speed of 3000 rpm for 15 minutes.

The data from this research were analyzed using one-way ANOVA. If between treatments there are significantly different results ($P<0.05$) then the analysis will continue with Duncan's multiple range test [24].

3. Results and discussion

3.1. Meat Chemistry

Providing fermented *Mangosteen* peel water extract (FMWE) in drinking water on the chemistry of broiler meat is presented in Table 2. The average water content of broiler meat given FMWE at P1, P2 and P3, was: 4.58%; 7.54%; and 8.12% significantly ($P<0.05$) lower compared to control (P0). The water content of meat in broiler groups P2 and P3, namely 3.10% and 3.71, was significantly different ($P<0.05$) lower than P1. The average ash content of broiler meat given FMWE in the P3 broiler group was 9.17% significantly ($P<0.05$) lower than P0.

The average fat content of broiler meat given FMEW in broiler groups P1, P2 and P3 was: 20.80%; 15.73%; and 12.80% significantly ($P<0.05$) higher than P0.The mean protein content of meat given FMWE in the P1 broiler group was 3.94% significantly ($P<0.05$) lower than P0.

Table 2 Effect of fermented *Mangosteen* peel water extract (FMWE) in drinking water on broiler meat chemistry

Variables	FMWE addition level in feed (%)				SEM
	0	1.5	3.0	4.5	
Water content (%)	62.19[c3)]	59.34[b]	57.50[a]	57.14[a]	0.26
Ash content (%)	1.09[b]	1.14[b]	1.14[b]	0.99[a]	0.02
Fat content (%)	3.75[a]	4.53[c]	4.34[b]	4.23[b]	0.04
Protein content (%)	28.46[b]	27.34[a]	27.58[ab]	27.78[ab]	0.29

Note: The mean with superscript (a,b) was significantly different (P≤0.05); SEM= Standard Errort of the Treatment Mean

The higher the FMWE concentration, the water content in the meat also decreases. As reported by [25], the decrease in water content in meat was caused by the addition of antioxidant sources in the feed. Antioxidants have the ability to bind water by breaking down ester bonds, so the more antioxidant sources you add, the lower the water content in the feed. *Mangosteen* peel contains bioactive compounds such as xanthones [26]and flavonoids [27] which act as antioxidants. The increase in water absorption in the extracellular and intracellular spaces in meat is caused by the absorption of water which is circulated through the blood throughout the body in a short time [28].

Providing FMWE in drinking water reduces the ash content of broiler meat. According to [29] stated that ash content is the amount of mineral content in meat and will increase with increasing age of the chicken. According to [30], fresh chicken meat has an ash content of 1.14%.

Fat content will increase as broiler age increases [31]. Meat fat can also be influenced by age, sex, feed and temperature [32]. Fat content has a negative correlation with protein content, if fat content increases then meat protein content decreases, and vice versa [33]. Meat water content is also negatively correlated with fat content.

The protein content of meat generally ranges from 19-22% [34]. The results of this study show that the higher level of FMWE administration can reduce the protein content of broiler meat insignificantly. This decrease was due to *Mangosteen* peel containing antimicrobials and antioxidants. Supported by [35] who reported that *Mangosteen* peel has bioactive compounds such as xanthones, as well as other compounds that act as antioxidants, antitumor, antiallergic, anti-inflammatory, antibacterial and antiviral. Antimicrobial compounds can inhibit microbial growth so as to reduce chemical damage to meat [36].

3.2. Blood Lipid Profile

The impact of providing FMWE via broiler drinking water from 0-4 weeks of age on the blood lipid profile of broilers is presented in Table 3. The average total blood serum cholesterol levels of broilers given FMWE in broiler groups P1, P2, and P3 were: 5.61%; 5.29%; and 4.11% not significantly (P>0.05) lower than the P0 or control broiler group.

Table 3 Effect of giving FMWE in broiler drinking water from 0-4 weeks of age on abdominal fat and blood lipid profile.

Variables	FMWE addition level in feed (%)				SEM
	0	1.5	3.0	4.5	
Total cholesterol (mg/dL)	156.38a	147.6a	148.1a	149.9a	3.83
HDL (mg/dL)	107.32a	116.05b	117.73b	118.21b	2.41
LDL (mg/dL)	18.12a	18.37a	18.59a	18.02a	1.04
Triglyserides (mg/dL)	146.69a	144.20a	143.29a	145.44a	2.67
Abdominal-fat (% body weight)	1.53a	1.24a	1.36a	1.26a	0.12

Note: The mean with superscript (a,b) was significantly different (P≤0.05); SEM= Standard Errort of the Treatment Mean

The average levels of HDL blood serum of broilers given FMWE in broiler groups P1, P2, and P3 were: 8.13%; 9.70%; and 10.15% significantly (P<0.05) higher than the P0 broiler group. The average levels of LDL blood serum of broilers given FMWE in broiler groups P1, P2 and P3 were: 1.38%; 2.59%; and 0.55% not significantly (P>0.05) lower compared

to the control (P0). The inclusion of FMWE in broiler drinking water had no significant effect (P>0.05) on broiler blood serum triglyceride levels. Likewise, the abdominal fat content of broiler chickens did not show any significant difference (P>0.05) with the presence of FMWE in their drinking water. More details are presented in Table 3.

Giving FMWE can reduce cholesterol levels in the blood. This is because it contains flavonoid compounds which can reduce cholesterol levels in the blood. Flavonoids are a large class of natural phenolic compounds. Phenolic compounds can prevent the formation of micelles in the intestine which involve bile salts, one of whose functions is to dissolve cholesterol in the intestine, so that ultimately the body's cholesterol will be reduced [37]. Flavonoid compounds can inhibit the absorption of cholesterol in the digestive tract, causing cholesterol to form complex compounds which are then excreted with the excreta [38].

The content of flavonoid compounds can reduce the absorption of cholesterol and bile acids in the small intestine, causing increased excretion through feces. *Mangosteen* peel also contains xanthones which function as antioxidants that inhibit the cholesterogenesis process [39]. According to [40], normal blood cholesterol levels in chickens range from 52-148 mg/dl. Based on this opinion, the cholesterol levels from the results of this study are classified as good, ranging between 141.75-159.93 mg/dl. Research by [41] which states that administering herbal extracts in drinking water at doses of 10-30ml can reduce cholesterol levels in the blood.

The results of this study show that giving FMWE in drinking water has a significant effect on High Density Lipoprotein (HDL) broilers. This is because the rind of the *Mangosteen* fruit contains xanthone compounds which play a role in increasing HDL [42]. Flavonoid compounds can inhibit the work of CETP (Cholesteryl ester transfer protein) thereby increasing HDL and reducing LDL [43].HDL and LDL have a positive correlation and are influenced by cholesterol levels in the blood. HDL is classified as a lipoprotein which functions as a means of transporting cholesterol cells from peripheral cells to liver cells and body glands [44].

Abdominal fat is closely related to carcass weight, if abdominal fat is high then carcass weight is low, because there is excess energy which results in the accumulation of fat in the animal's body [45]. Septinar et al. [7] reported that other factors influence body fat content, namely breed, breed, gender, age and housing system. The percentage of abdominal fat will also increase if body weight increases [46].

Giving FMWE at a level of 1.5-4.5% can produce lower abdominal fat compared to giving without FMWE. This is due to the presence of saponins and flavonoids in *Mangosteen* peel. Saponins have the ability to block the transport of nutrients including fat. Saponin is able to inhibit the way pancreatic lipase works, thus damaging the fat oxidation process which causes reduced fat formation [47]. The flavonoid content also plays a role in reducing fat. In line with [48] said that flavonoids can slow down the activity of the Fatty Accid Synthaase (FAS) enzyme which plays a role in fat metabolism, thereby inhibiting the formation of fatty acids.

4. Conclusion

It can be concluded that the administration of 4.5% water extract of fermented *Mangosteen* peel in broiler drinking water significantly reduces the water content and ash content of meat. On the contrary, it significantly increased the meat fat content and blood serum HDL levels of broilers.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare that there is no conflict of interest with any party regarding the material discussed in the manuscript.

Statement of ethical approval

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

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