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Meat Fatty Acid Composition and Malondialdehyde Concentration of Dried Star Gooseberry Leaf Extract for Duck Feed

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Abstract: Local ducks have good potential to be developed as a producer of meat and eggs as a source of animal protein. However, compared to other poultry, duck meat has relatively high fat and cholesterol contents, which are not preferred as they may have harmful effects on health. Additionally, excessive meat fat content can also cause a rotten smell to the meat. Containing carotene, flavonoids, tannins, and antioxidants, star gooseberry (*Sauropus androgynus*) leaves are potential to be used a feed additive in poultry ration. This study aimed to assess the effects of the inclusion of dried star gooseberry leaf extract (DSGLE) meal in rations on the composition of fatty acids, malondialdehyde (MDA), and cholesterol of local duck meat. One-hundred local ducks were randomly allocated into 5 treatments and 5 replicates in a completely randomized experimental design. Treatments consisted of rations containing 0, 0.5, 1.0, 1.5, and 2.0% DSGLE. Measurements were taken on meat fat content, MDA contents, cholesterol level, and fatty acid composition. Results showed that treatments significantly affected meat fat content, fatty acid composition, cholesterol level, and MDA content. It was concluded that feeding ducks with DSGLE meal reduced meat fat and cholesterol levels, improved meat fatty acid composition and protected the meat from oxidative damage. This study reinforced the benefits of using phytochemicals in diets in improving the production performance and product quality of poultry animals. Results of this study uncovered another way to produce healthier duck meat containing less fat and having less off-odor.

Keywords: *Sauropus androgynus*, cholesterol, antioxidation, meat quality.

鸭饲料用干星醋栗叶提取物的肉脂肪酸组成和丙二醛浓度

摘要：本地鸭子有很大的潜力被开发为肉和蛋的生产者，作为动物蛋白的来源。然而，与其他禽肉相比，鸭肉的脂肪和胆固醇含量较高，可能对健康有害，因此不宜食用。此外，过多的肉脂肪含量也会使肉产生腐烂的气味。星醋栗(雌雄蜥)叶含有胡萝卜素、类黄酮、单宁和抗氧化剂，有可能用作家禽日粮中的饲料添加剂。本研究旨在评估日粮中加入干星醋栗叶提取物(DSGLE)粉对当地鸭肉中脂肪酸、丙二醛(丙二醛)和胆固醇成分的影响。在完全随机的实验设计中，将 100 只本地鸭子随机分配到 5 个处理和 5 个重复中。治疗包括含有 0、0.5、1.0、1.5 和 2.0% DSGLE 的日粮。对肉脂肪含量、丙二醛含量、胆固醇水平和脂肪酸组成进行了测量。结果表明，处理显着影响肉脂肪含量、脂肪酸组成、胆固醇水平和丙二醛含量。得出的结论是，用 DSGLE 膳食喂养鸭子可降低肉脂肪和胆固醇水平，改善肉脂肪酸组成并保护肉免受氧化损伤。该研究强调了在日粮中使用植物化学物质对提高家禽动物的生产性能和产品质量的好处。这项研究的结果揭示了另一种生产更健康的鸭肉的方法，它含有更少的脂肪和更少的异味。

关键词：雌雄蜥, 胆固醇, 抗氧化, 肉品质.

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1. Introduction

Until now, the duck meat production has not been able to match that of purebred chickens. The increase in duck meat production is not as large as that of broiler meat. As livestock products play an important role in improving people’s welfare, producers are required to produce meat that is not only tasty and tender but also healthy with high nutrient and low fat and cholesterol contents. One of the causes of low consumer demand for duck meat is its relatively high fat and cholesterol content.

It is commonly believed that excessive levels of cholesterol in the blood may increase the risk of accumulation or deposition of cholesterol on the walls of arteries, which may lead to the development of atherosclerosis, coronary heart disease (CHD), and stroke [1]. It has been a big challenge for the poultry industry to produce poultry products containing high unsaturated fatty acids and low cholesterol. The use of feed additives containing many kinds of active substances is a promising answer to this challenge. Star gooseberry leaves are known to contain chemical compounds including phytosterols, tannins, saponins, flavonoids, alkaloids, saponins, sterols, amino acids, proteins, carbohydrates, vitamins, and minerals [2]-[3]. Star gooseberry was found as one of 24 Indonesian native vegetables with the highest content of kaempferol, an antioxidative flavonoid polyphenol [4].

The use of star gooseberry leaves in diets and its effects on the performance and quality of products of ruminants and poultry have been the subject of many studies. The research results in [5] showed that feeding fish with an extract of star gooseberry leaves significantly increased growth and feed consumption but decreased feed conversion. Does fed star gooseberry leaves produced colostrum milk with higher protein content than that of does control group (5.41 vs 4.05%) [6]. However, supplementation of these leaves in Bali cow diets failed to improve milk yield and quality [7]. More extensive studies have been conducted on the use of star gooseberry leaf meal in rations of poultry animals. Feeding star gooseberry leaf meal was found to increase fertility and hatchability rates [8] and egg quality without hampering egg production in quails [9]. Star gooseberry leaf meal inclusion in rations was found to increase body weight [10], reduce feed intake, improve feed conversion ratio, lower carcass fat content [11] in broiler chickens, improve meat tenderness in local chickens [12], increase egg production in layer chickens [13], and improve the quality of yolk color and aroma of duck eggs [14].

Instead of the positive effects of the star gooseberry leaves on improving the production performance of various kinds of animals attributable to the antimicrobial and antioxidative properties of secondary metabolite contents, adverse effects on growth were observed. This was found in broiler chickens fed star gooseberry leaves, which were given in the form of an unprocessed ingredient [15]. High content of tannins was suspected to be the cause of this phenomenon as they are known as anti-nutritional compounds, which are detrimental to feed intake and digestive processes in ruminant and monogastric animals particularly when it is used inappropriately and at high concentrations [16]-[17]. The application of treatments including fermentation and extraction of gooseberry leaves to overcome the problem has been studied [15], [18]-[20]. Based on this notion, a study on the effects of DSGLE on the fatty acid composition of duck meat was worthwhile to conduct.

2. Materials and Methods

2.1. Animals

One-hundred local ducks aged 9 months with an average initial body weight of 1406.25 ± 211.32 g were used. The ducks were raised in 25 battery cages (4 ducks per cage) and randomly allocated into 5 treatments in a completely randomized design with 5 replicates.

2.2. Star Gooseberry Leaf Extract

DGLSE was prepared based on the procedures used in [21]. Fifty grams of star gooseberry leaves were weighed and soaked in 300 ml ethanol (96%). The mixture was stirred slowly while it was heated in a water bath at 60°C for 2 hours. The mixture was then filtered, and the filtrate was evaporated until its volume reduced to 100 ml. Ninety grams of corn meal was added to the filtrate and mixture was stirred slowly and dried in an oven at 50-60°C for 90 minutes.

2.3. Treatment Rations

Basal rations were formulated from corn meal, rice bran, soybean cake meal, fish meal, premix, CaCO3, CPO, and DCP. Treatment rations consisted of basal ration and the inclusion of DSGLE by 0% (R0), 0.5% (R1), 1.0% (R2), 1.5% (R3), and 2.0% (R4). Feed composition and nutrient content of the treatment ratios are listed in Table 1.

Table 1 Feed composition and nutrient content of treatment rations

	Treatment				
	R0	R1	R2	R3	R4
Feed/nutrient	**Amount (%)**				
Corn meal	61.00	61.00	61.00	61.00	61.00
Rice bran	8.00	8.00	8.00	8.00	8.00
Soybean cake meal	11.50	11.50	11.50	11.50	11.50
Fish meal	10.50	10.50	10.50	10.50	10.50
Premix	1.00	1.00	1.00	1.00	1.00
CaCO3	5.00	5.00	5.00	5.00	5.00
Crude palm oil	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	2.50	2.50	2.50	2.50	2.50
DSGLE	0.00	0.50	1.00	1.50	2.00

Continuation of Table 1					
Dry matter	89.12	89.08	89.04	89.00	88.97
Crude protein	16.31	16.35	16.40	16.44	16.49
Fiber	4.51	4.51	4.51	4.51	4.51
Crude fat	3.68	3.69	3.70	3.72	3.73
Ash	8.08	8.09	8.10	8.10	8.11

Notes: RO: 0% DSGLE, R1: 0.5% DSGLE, R2: 1.0% DSGLE, R3: 1.5% DSGLE, R4: 2.0% DSGLE

2.4. Meat Fat, Cholesterol, and MDA Contents

The meat fat content was determined by referring to the method developed by [22]. Dried and homogenized meat samples were washed with ether in a Soxhlet extractor for 16 hours. The lipid extract was then evaporated at 95-100°C and weighed. Fat content was the difference in the weight of the samples before and after extraction.

Meat cholesterol was analyzed following the instructions of [23]. Meat samples of 0.1 mg were dissolved in 12 ml of alcohol-ether solution (3:1) in a mortar and ground until a homogenous mixture was obtained. The mixture was transferred into tubes and centrifuged at 3000 rpm for 15 minutes. The supernatant was heated on a hot plate and the dried sample was dissolved in 5 ml chloroform and vortexed until it became homogenous. Anhydrous acetic acid of 2 ml was then added to the solution before it was further homogenized using a vortex. One milliliter of concentrated sulfuric acid was added to the solution before it was allowed to stand for 15 minutes in a dark room. Finally, the absorbance was measured using a UV spectrophotometer at a wavelength of 430 nm.

MDA levels were determined by referring to the method of [24]. MDA level was quantified based of the ability of it to react with thiobarbituric acid (TBA). Every one molecule of MDA reacted with two molecules of TBA. The reaction was indicated by the appearance of pink pigment with maximum absorption at 532-538 nm. Frozen meat (-200°C) was thawed and used as a sample. A cold meat sample of 1.25 g was placed in an Erlenmeyer flask and chopped by using a syringe needle. Cold phosphate-buffered saline (2.5 ml, 5°C) containing 11.5 g/l potassium chloride (pH 7.4) was added in. The mixture was centrifuged at 4000 rpm for 10 minutes. Clear supernatant of 1 ml was taken and mixed with a mixture solution of cold 0.25 N (2.23 ml concentrated chloride acid/100 ml) chloride acid containing 15% (w/v) trichloroacetic acid, 0.38% thiobarbituric acid, and 0.5% butylated hydroxytoluene. The mixture was heated in an incubator at 80°C for an hour before it was cooled with running water and centrifuged at 3500 rpm for 10 minutes. The absorbance of this centrifuged supernatant was measured at 532 nm wavelength.

Fatty acid compositions were determined using a method developed by [25]. Meat sample of 100 g was extracted and homogenized using chloroform and methanol solution (2:1). NaCl 1.5% solution was then added in to form methyl esters, which were further injected into a gas chromatography by using a GC-2010 Plus-Shimadzu AOC 20i autoinjector, SP®-2560 capillary columns L × I.D 100 m × 0.25 mm, df 0.20 μm, Supelco, Bellefonte, PA. The initial temperature was 70°C and it was increased gradually to 175°C within 27 minutes. The temperature was further elevated by 4°C per minute until it reached 215°C. This temperature was then held for 31 minutes.

Fatty acids were identified by comparing the retention time of sample methyl esters to standard fatty acid methyl esters (FAMEs) including C4-C24 (FAME mix Sigma®), vaccenic acid C18: 1 trans-11 (V038-1G, Sigma®), C18: 2 trans-10 cis-12 (UC 61 M 100 mg), CLA C18: 2 cis-9, trans-11 (UC 60 M 100 mg) (Sigma®), and tricosanoic acid (Sigma®). Fatty acids were measured by normalizing the area under the methyl ester curve using 2.42, Shimadzu GC-2010 software. The amount of fatty acid was defined as the percentage of total FAMEs.

3. Results and Discussion

An indication of the occurrence of oxidation in meat can be detected through the measurement of fat content, fatty acid composition, and levels of MDA formed. Increased fat content in duck meat is mainly caused by increases in triglyceride, saturated fatty acid, unsaturated fatty acid, and intramuscular fat contents. Therefore, feed fatty acid composition plays an important role in modifying meat fat content [26].

It was revealed that the value of meat saturated fatty acids (SFA) was not as large as that of polyunsaturated fatty acids (PUFA) (Table 2). This was not unexpected as, compared to red meat, poultry meat contains less fat and more unsaturated fatty acids than saturated ones [27]-[29]. However, PUFAs in poultry meat are more susceptible to oxidation [30].

Ducks fed on DSGLE were found to have meat containing higher PUFAs. This finding indicated that the antioxidant activity of DSGLE leaves could protect PUFAs from oxidation. Star gooseberry leaves contain high active phenolic compounds, including quercetin and kaempferol, having antioxidative properties [4]. Diet supplementation with quercetin was found to reduce lipid oxidation in broiler meat [30]-[31]. In this study, this notion was supported by the fact that meat MDA level was significantly lower (0.11 g/100 g) in ducks fed the highest level of DSGLE (Table 3). MDA is a by-product of PUFA peroxidation and an indicator of oxidative stress [32]. In addition, extracts of star gooseberry leaves were proved to have 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities [33] [34]. Lower meat MDA levels were also found in ducks treated with leaf meals of *Pluchea indica Less* (*beluntas*) and *Cosmos caudatus* (*kenikir*) which are known to contain antioxidative phenols [35].

Table 2 Meat fatty acid composition of duck fed DSGLE (%)

Fatty acid	R0	R1	R2	R3	R4
Lauric	0.18 ± 0.06^b	0.15 ± 0.06^b	0.15 ± 0.04^b	0.08 ± 0.02^a	0.073 ± 0.01^a
Myristic	0.49 ± 0.05^b	0.47 ± 0.05^b	0.46 ± 0.07^{ab}	0.42 ± 0.04^a	0.41 ± 0.03^a
Palmitic	17.10 ± 1.97^b	16.67 ± 0.92^b	15.01 ± 0.76^a	15.30 ± 0.78^a	16.32 ± 0.53^b
Stearic	3.72 ± 1.08	4.14 ± 1.36	3.52 ± 0.81	3.45 ± 0.67	3.43 ± 0.56
SFA	21.48 ± 2.59^a	21.42 ± 2.18^a	19.14 ± 0.95^b	19.24 ± 0.81^b	20.23 ± 0.90^{ab}
Palmitoleic	1.67 ± 0.15^d	1.17 ± 0.09^a	1.42 ± 0.12^b	1.56 ± 0.18^{bcd}	1.47 ± 0.07^{abc}
Elaidic	0.41 ± 0.07	0.40 ± 0.04	0.36 ± 0.06	0.41 ± 0.09	0.39 ± 0.04
Oleic	25.73 ± 2.24^{ab}	24.30 ± 2.15^a	25.11 ± 1.62^a	26.74 ± 1.70^b	25.25 ± 1.14^{ab}
Linoleic	9.46 ± 1.02^a	11.18 ± 1.40^b	11.73 ± 1.22^{bc}	11.08 ± 1.35^b	11.65 ± 1.00^c
PUFA	37.27 ± 2.84^a	37.06 ± 2.58^a	38.61 ± 1.71^{ab}	40.04 ± 1.68^b	40.50 ± 1.52^b

Notes: Different superscripts in the same rows indicate significant differences (P<0.05). RO: 0% DSGLE, R1: 0.5% DSGLE, R2: 1.0% DSGLE, R3: 1.5% DSGLE, R4: 2.0% DSGLE, SFA: saturated fatty acids, PUFA: polyunsaturated fatty acids.

Meat fat and cholesterol contents were significantly lower in ducks fed on DSGLE (Table 3). These results agreed with what were found in other studies. In broiler chicken, the use of DSGLE resulted in meat with lower fat (1.44 vs. 5.95 g/100 g) and cholesterol (0.10 vs. 0.15 g/100 g) contents [15]. Ducks given star gooseberry leaf meal by 7.5% were found to have lower egg cholesterol levels (12.82 mg/g egg yolk) than those in the control group (15.35 mg/g egg yolk) [36]. These fat and cholesterol reducing effects might be attributable to groups of phenolic compounds, tannins, flavonoids, saponoids, triterpenoids, and alkaloids, which can reduce fat [37]. Feeding ducks with various plant leaves rich in fat-reducing secondary metabolites was found to significantly lower the fat and cholesterol meat content. Bay leaf supplementation with 9% lowered meat fat contents from 2.59 to 1.22 and 1.44 to 1.25% and meat cholesterol levels from 1.17 to 1.03 and 1.25 to 1.02 mg/g in Mallard and Muscovy ducks, respectively [38]. In another study, in male Tegal ducks aged 9 weeks, feeding diets supplemented with 12% bread fruit leaf meal decreased meat fat and cholesterol contents from 5.56 to 5.05% and from 174.82 to 154.88 mg/100 g, respectively [39].

Table 3 Meat fat, cholesterol, and MDA contents of duck fed DSGLE (g/100 g)

	R0	R1	R2	R3	R4
Fat	27.33 ± 1.21^b	26.27 ± 1.38^b	27.07 ± 1.20^b	26.75 ± 1.14^a	25.22 ± 1.40^a
MDA	0.36 ± 0.22^c	0.36 ± 0.07^c	0.25 ± 0.08^{bc}	0.21 ± 0.06^{ab}	0.11 ± 0.06^a
Cholesterol	83.5 ± 8.3^a	83.69 ± 7.34^a	83.46 ± 7.34^a	77.10 ± 7.81^{ab}	75.56 ± 6.34^b

Notes: Different superscripts in the same rows indicate significant differences (P < 0.05). RO: 0% DSGLE, R1: 0.5% DSGLE, R2: 1.0% DSGLE, R3: 1.5% DSGLE, R4: 2.0% DSGLE, MDA: malondialdehyde

4. Conclusion

The use of star gooseberry leaves extracted by using ethanol (DSGLE) in the ration lowered duck meat fat and cholesterol levels, improved meat fatty acid composition, and protected the meat from oxidative damage.

This study reinforced the benefits of using phytochemicals in diets in improving the production performance and product quality of poultry animals. Results of this study revealed a way to produce healthier duck meat containing less fat and having less off odor.

Nonetheless, this study was conducted on a laboratory scale using a limited number of animals. Studies in larger farming scale involving a higher number of animals need to be conducted for more conclusive results. Economic aspects of the use of star gooseberry leaf extract in duck production and poultry production in general should be assessed. An investigation on the best feeding manner of star gooseberry leaves in poultry also deserves to be conducted.

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