Effect of Diet Containing Gracilaria Sp. Waste and Multi-Enzyme

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Effect of Diet Containing *Gracilaria* Sp. Waste and Multi-Enzyme Additives on Blood Lipid Profile of Local Duck

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Abstract. This study was aimed to evaluate the effect of diet containing waste of seaweed *Gracilaria* sp. on local lipid profiles of duck blood including cholesterol, triglycerides, low density lipoprotein (LDL), and high density lipoprotein (HDL). The material in this study were 72 female Tegal ducks aged 22 weeks with 1,318 ± 121 g average body weight, diets containing *Gracilaria* sp. waste (GW) with 18% protein and 2900 kcal/kg metabolic energy and 150 g/ton feed commercial multi-enzyme (ME). Data were subject to Completely Randomized Design with 6 treatments, (T_0) Basal Diets, (T_{0+}) Basal Diets with ME, (T_1) Diets with 10% GW, (T_{1+}) Diets with 10% GW + ME, (T_{2+}) Diets with 12.5 % GW + ME, and (T_{3+}) Diets with 15% GW + ME. The treatment was given for 12 weeks and blood sample was taken on the last week. The serum was separated and analyzed for blood lipid profiles using CHOD-PAP. Results showed that diet with 12.5%GW plus multi-enzyme additives significantly (P<0.05) affected blood lipid profiles of local duck, tended to lower triglycerides and LDL Cholesterol, but increase blood HDL levels.

Key words: blood lipids, local ducks, *Gracilaria* sp. waste, multi-enzyme

Abstrak. Penelitian ini bertujuan untuk mengevaluasi pengaruh ransum yang mengandung limbah rumput laut *Gracilaria* sp. Terhadap profi lemak darah itik local antara lain kolesterol, trigliserida, *low density lipoprotein* (LDL), dan *high density lipoprotein* (HDL).Materi yang digunakan dalam penelitian ini adalah itik Tegal betina umur 22 minggu dengan bobot badan rata-rata 1.318±121g sebanyak 72ekor, ransum mengandung limbah *Gracilaria* sp. (LG) dengan protein 18% dan energy metabolis 2900 kcal/kg dan multienzim (ME) komersial dengan dosis penggunaan 150 g/ton pakan. Penelitian menggunakan Rancangan Acak Lengkap dengan 6 perlakuan yaitu (T_0) Ransum Basal, (T_{0+}) Ransum Basal dengan ME, (T_1) Ransumdengan 10% LG (T_{1+}) Ransumdengan 10% LG + ME, (T_{2+}) Ransum dengan 12,5% LG + ME (T_{3+}) Ransum dengan 15% LG + ME. Perlakuan dilakukan selama 12 minggu dan pengambilan sampel darah pada minggu terakhir. Serum dipisahkan dan dianalisis profile mak darah menggunakan metode CHOD-PAP. Hasil menunjukkan bahwa pemberian ransum mengandung LG 12,5% ditambah aditif multienzim berpengaruh nyata (P<0,05) terhadap profil lemak darah itik lokal, cenderung menurunkan Trigliserida, Kolesterol LDL sebaliknya meningkatkan kadar HDL darah.
Kata kunci :lemak darah, itik lokal, limbah *Gracilaria* sp., multienzim

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Introduction

Ducks have a great opportunity to be continuously farmed for their better endurance and adaptability than other poultry. Research was aimed to increase productivity associated with health properties of blood chemistry becomes particularly important indicator parameter of diet treatment. Influential factors of fat deposition in livestock products are genetic factors, nutrients, medicines and diet. Blood is vital fluid that acts as nutrients transport throughout the body to perform its functions. Blood chemistry is closely related to physiological and biochemical processes in the body. The ideal blood lipid profile including cholesterol, triglycerides, low LDL and high HDL will be an indicator of biochemical processes in the body of cattle running normally so it can yield good quality product.

High price of feed and high competition of farmers with feed processing industry to get quality feed ingredients constrain farmers to improve their maintenance patterns. It is necessary to make abundant alternative feed ingredients with economic value and high nutrition. Modification of waste-original feed into alternative is to increase productivity and quality of livestock products. One example is waste of seaweed cultivation of *Gracilaria* sp. as one of the potential of maritime countries such as Indonesia.

Ministry of Internal Affairs (2013) reported that Indonesian seaweed production reached 10 million tons in 2014 and continued to increase. Approximately 65-70% of the total cultivation of seaweed are sorted-waste that do not have commercial value. Seaweed *Gracilaria* sp. waste consists of the dull-colored plants and is sorted from seaweed cultivation (Alamsjah et al., 2011; Mustaqim et al., 2014). *Gracilaria* sp. waste will be very useful when utilized in order to reduce environmental damage, which can be used to feed the cattle.

Patel and Goyal (2011) stated that the nature of seaweed wastes is able to reduce hyperlipidemia. Antioxidant compounds contained in *Gracilaria* sp. waste can reduce blood lipid. Its cell wall is composed of calcium carbonate ($CaCO3$), cellulose, and photosynthetic products such as carrageenan and agar (Suparmi and Sahri, 2009). *Gracilaria* sp. waste contains coarse fiber cellulose and polysaccharides that is difficult to digest by enzymes in digestive tract. Cellulose in *Gracilaria* sp. waste included in *Xylooligosaccharides* in which β (1-4) glucosidal binds (Madhukumar and Muralikhrishna, 2010). Wu et al. (2005) and Hu et al. (2006) also mentioned other content, *Algal-oligosaccharides lysate* (AOL) and *Neoagaro-oligosaccharides* (NAOS). Analysis on complex structure of seaweed molecule to obtain the active ingredients in seaweed showed that polysaccharides contained in *Gracilaria* sp. is sulfated polysaccharide. Sulfated polysaccharide is rich of *fucoidan*. It has one type of biological sugars namely monomer *fucose*, and *galactose*, *xylose*, *mannose* and *glucoronic acid* residue (Lim et al., 2014). The purification process of polysaccharide from seaweed produces 9.87 mg / g *phenol* and 20.16 mg / g *flavonoids* as antioxidants (Woo et al., 2012; Lim et al., 2014; Imjongjairak et al., 2015; Chan et al., 2015).

Previous research on waste of seaweed was conducted to improve the nutrient quality through fermentation process but only 10% the most (Yunita et al., 2015). Although the fermentation process can increase the nutrient content of feed and lower coarse fiber content, the protein in fermented feed is single cell protein formed from microbes. Single cell protein is the limiting factor because it causes metabolic disturbances in the body of cattle (Sinurat et al., 2003). In addition, the fiber content of the diet is not only influenced by the fiber content in *Gracilaria* sp. waste, but also by coarse fiber content of other substances composition in diets. Application of fermentation technology on livestock becomes less practical and requires high costs. Therefore, this study needs to find a more applicable method in community by using commercial multi-enzyme added to diet that already contains*Gracilaria* sp. waste Multi-enzyme is expected to increase the nutritional value of seaweed waste and other feed ingredients in the diet.

Materials and Method

This study used 72 female Tegal ducks aged 22 weeks with 1,318±121 g average body weight. Feed contained 18% protein and 2,900 kcal/kg metabolic energy. The composition of the feed material is presented in table 1. *Gracilaria* sp. waste was given in the form of flour after the waste was washed to remove salt content, sun-dried to obtain 15% moisture (Ventura et al., 1994) then milled into flour. Multi-enzyme additive used was Allzyme SSF from Alltech Ltd. consisted of Non Starch Polysaccharides breaker enzyme or NSP (*cellulase*, *xylanase*, *pectinase*, *β-glucanase*), *amylase*, *phytase* and *protease* enzyme at a dose of 150 g/ton of feed.

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Table 1. Ingredients and Nutrient Compositions of the Experimental Diet

Feed material	T0 (0%)	T1 (10%)	T2 (12,5%)	T3 (15%)
Corn	54.8	52.7	51.0	49.0
Gracilaria sp.	0	10.0	12.5	15.0
Soybean Meal	17.2	19.7	19.7	20.0
Oil	0.8	0.2	0.2	0.4
Ricebran	14.7	4.8	4.0	3.0
Fish Meal	7.0	8.5	8.5	8.5
$CaCO_3$	1.9	1.0	1.0	1.0
Premix	1.0	1.0	1.0	1.0
Methionin	0.4	0.4	0.4	0.4
Lysin	0.7	0.7	0.7	0.7
Brotiacostulla	1.0	1.0	1.0	1.0
Total	100	100	100	100
Nutrient Composition(%)				
EM (kkal/kg)*	2902.29	2923.22	2909.41	2907.28
Crude Protein (%)*	18.05	18.17	18.15	18.25
Crude Fat (%)*	8.05	7.52	7.26	7.61
Crude Fiber (%)*	6.63	5.47	5.56	5.59
Methionin (%)**	1.36	0.75	0.74	0.74
Lysin (%)**	0.63	1.27	1.26	1.25
Arginin (%)**	1.24	1.24	1.22	1.20
Calcium (%)*	2.78	2.02	2.03	2.03
Phospor (%)*	0.73	0.69	0.89	0.68

T1–T3: Treatment with *Gracilaria* sp. waste (GW)

T0: basal diet.

T1, T2, and T 3: 10% GW, 12,5% GW, and 15% GW, respectively.

Energy metabolism (EM) was measured based on the Balton formula.

Nitrogen free extract = 100 – (% water + % ash + % crude protein + % crude fat + % crude fiber).

Energy metabolism (EM) = 40.81 {0.87 (crude protein + 2.25 crude fat + nitrogen free extract + 4.9}.

The study was subject to Completely Randomized Design with 6 treatments and 4 replicates, each consisted of three ducks, 60 ducks in total. Study treatment consisted of (T_0) Diets Basal, (T_0+) Diets Basal with ME, (T_1) Diets with 10% GW, (T_{1+}) Diets with 10% GW + ME, (T_{2+}) Diets with 12.5% GW + ME, and (T_{3+}) Diets with 15% GW + ME.

The study lasted for 12 weeks with 2-week adaptation period. Diet was given two times a day and drinking water was provided ad libitum. Blood sampling was performed in the last week of treatment period, taking 3 ml of blood from brachial vein using injection syringe, kept in the tube and let stand to separate serum. Serum was taken using a pipette and stored in Eppendorf tubes for further blood lipid profile test including cholesterol, triglycerides, LDL, and HDL using CHOD-PAP method (*Cholesterol Oxidase Phenyl peroxidase Amino Phenozon phenol*) based on DSI (2005).

Data obtained were tabulated and subjected to analysis of variance (ANOVA) then tested further using the LSD (*Least Significant Different*) test. Significance level was determined at P<0.05.

Results and Discussion

The use of diet containing *Gracilaria* sp. waste (GW) with multi-enzyme showed significant effect (P <0.05) on the local duck blood lipid profile (Table 2). The lowest blood cholesterol was in T_2+ or GW 12.5% plus multi-enzyme. This was likely due to fiber content and the anti-cholesterol active compounds in *Gracilaria* sp. waste.

Woo et al., (2012) added that the extraction of the seaweed species *Gracilaria* sp. produced antioxidant compounds such as phenol components especially flavonoids. These compounds play a role in the inhibition of fat accumulation because it is hipolipidemia. Phenol can lower blood cholesterol, according to Gani et al. (2013), phenolic compounds can reduce plasma cholesterol levels by inhibiting the absorption of cholesterol by the intestine and increases the formation reaction of bile acids by cholesterol to be excreted through feces. Antioxidants prevent fat oxidation by binding metal ions as oxygen antidote, then eliminating oxygen radicals so it remains antioxidants together with unsaturated fatty acids (Oktaviani, 2014). *Gracilaria* sp. waste also contains essential fatty acids in a proportion amount required by the body namely fatty acid omega 6 and 9 (Table 3).

The degree of saturation and the amount of fatty acids in the diet can affect the concentration of plasma lipoproteins and cellular cholesterol uptake. Unsaturated double chain fatty acids will increase the hydrolysis of cholesterol (Iriantiet al., 2005 and Silva et al., 2013).

Fibers in *Gracilaria* sp. according to Zhang et al. (2003) acted as an anticoagulant, anti hiperlipidemia, antitumor and antiviral. This is due to the presence of secondary metabolites compound that is bioactive named fucoidan (Bilanet al., 2007 and Shevchenko et al., 2007).

Table 2. The Average Blood Lipid Content of Local Ducks

Parameters	Treatment					
	T0	T0+	T1	T1+	T2+	T3+
Cholesterol (mg/dl)	177.02±21.66[ab]	220.77±92.08[c]	190.40±53.79[bc]	178.44±50.01[ab]	108.24±32.10[a]	115.22±48.87[ab]
Trigliseride (mg/dl)	92.54±18.30[a]	168.96±100.87[b]	122.87±56.03[a]	117.89±45.97[a]	80.20±22.88[a]	101.88±44.94[a]

a, b, c : Means in the same row with different superscripts differ significantly (P < 0.05) according to LSD (*Least Significant Different*)test.
T1–T3 :Treatment with *Gracilaria* sp. waste (GW)
T0 : basal diet; T0+ : basal diet + multienzyme supplemented
T1+, T2+, and T3+: 10% GW, 12,5% GW, and 15% GW + multienzyme supplemented

Tabel 3. Fatty Acid Content in *Gracilariasp.*Waste

No	Fatty Acid	Content (%)	Formulation
1.	Capric Acid	0.37	C_{10}:0
2.	Lauric Acid	11.11	C_{12}:0
3.	Myristic Acid	8.04	C_{14}:0
4.	Palmitic Acid	67.44	C_{16}:0
5.	Oleic Acid	6.99	C_{18}:1ω9
6.	Stearic Acid	1.75	C_{18}:0
7.	Linoleic Acid	4.3	C_{18}:2ω6

Based on the test results of the Integrated Research and Testing Laboratory of the University of Gajah Mada, Yogyakarta (2015)

Fucoidan included in the sulfate polysaccharides has anti-cholesterol effect, by inhibiting the release of glucose to the blood. Diet containing *Gracilaria* sp. waste will turn out to be condensed in the digestive tract due to the fiber contained in the *Gracilaria* sp. waste as polysaccharides such as fucoidan, alginate, seaweed and carrageenan. Fucoidan complex compounds contained in *Gracilaria* sp. waste consists of a xylose fraction (Xylo-oligosaccharides), mannose, galactose, glucose and several other polysaccharides (Bilan et al., 2010). As soluble fiber, the fiber content in seaweed leads to viscous gel in the digestive tract. Some fiber will be fermented in the digestive tract, but mostly will be absorbed directly without being digested (Ramnani et al., 2011). The use of multi-enzyme in this study was aimed to replace fermentation technology. Multi-enzyme used consists of fermented substance by Aspergillus niger packaged in the Solid State Fermentation (SSF) product, comprising breaker enzyme of Non Starch Polysaccharides (NSP) namely Cellulase, Pectinase, Mananase, and β glucanase. These four types of enzymes will work on the substrate in feed materials making up the diet mainly on *Gracilaria* sp. waste componentso that the active compound can work in the digestive tract. Other enzymes like amylase, protease and phytase will help to improve the digestibility of other feed ingredients. Enzymes help lower gel viscosity in digestive tract, improve the entrance of endogeneous enzyme to the reserve nutrients, and set the trapped substances free. Diets containing *Gracilaria* sp. waste and multi-enzyme consumed by ducks will automatically work on an appropriate atmosphere for the performance of the enzyme.

A diet containing *Gracilaria* sp. waste and multi-enzyme show the effect on blood triglyceride levels of local ducks. Effect of diet is evident in the control treatment with multi-enzyme (T_{0+}). This is because the digestibility of feed components, especially high fat as the role multi-enzyme. The lowest blood triglyceride levels was in 12.5% seaweed waste (T_{2+}). Wijesekara et al. (2011) reported on polysaccharides of the sea product dietary fiber types such as seaweed are containing of hipolipidemia product that can lower serum fats such as triglycerides and cholesterol as well as improve LDL and HDL. Cellulose that forms cell wall of *Gracilaria* sp. waste is broken down with the aid of multi-enzyme so that the active compound may work in the body of ducks.

Polyphenol compounds in the *Gracilaria* sp. waste works by inhibiting the activity of the enzyme HMG-CoA reductase that is an essential enzyme in cholesterol synthesis. Disturbances in the activity of this enzyme would lead to the decreased number of mevalonic acid as cholesterol precursor (Reddy et al., 2014). Barriers of HMG-KoA enzyme in liver stimulates LDL receptors thereby increasing the clearance of LDL from the bloodstream and lowers blood cholesterol levels. As a result, of disturbance in cholesterol biosynthesis, hence the formation of VLDL in the liver was inhibited so that blood triglyceride levels be decreased due to decreased production of VLDL which serves as a carrier of triglycerides in the blood vessels (Park et al., 2015).

LDL and HDL level of local ducks fed with *Gracilaria* sp. waste also changed. Further LSD test showed apparent discrepancies between T_1 with T_{1+} (diet with 10% *Gracilaria* sp. waste and 10% *Gracilaria* sp. waste plus multi-enzyme, respectively) and treatment T_{2+} (diet with 12.5% *Gracilaria* sp. waste). The basal diet containing multi-enzyme shows equal average LDL to that with 12.5% and 15% GW (Table 4). Diet with GW had the same effect as basal diet with only multi-enzyme.

Feed containing *Gracilaria* sp. waste could lower LDL levels. While the added multi-enzyme led to the lowest decreasing level of blood LDL in the *Gracilaria* sp. waste 12.5% with multi-enzyme. The average blood LDL level of local

Table 4. The Average of Blood Lipoprotein Content of Local Ducks

Parameters	Treatment					
	T_0	T_{0+}	T_1	T_{1+}	T_{2+}	T_{3+}
LDL (mg/dl)	103.32±10.74^b	81.22±31.82^a	108.09±21.17^b	106.44±33.73^b	62.02±32.92^a	95.24±13.35^a
HDL (mg/dl)	33.92±8.19^b	40.95±12.78^b	44.77±5.21^b	46.47±8.64^b	46.52±14.17^b	52.57±16.29^a

a, b: Means in the same row with different superscripts differ significantly (P < 0.05) according to LSD (*Least Significant Different*) test.

T1–T3:Treatment with *Gracilaria* sp. waste (GW)

T0: basal diet; T0+: basal diet + multi-enzyme supplemented

T1+, T2+, and T3+: 10% GW, 12,5% GW, and 15% GW + multi-enzyme supplemented

duck tended to decrease with the increasing seaweed *Gracilaria* sp. waste plus multi-enzyme although not significantly different. It showed favorable results as well as HDL blood levels that tended to rise. Highest level of HDL blood was obtained through diet with 15%*Gracilaria* sp. waste plus multi-enzyme.

It is important to know whether the cholesterol distributed in plasma lipoprotein is mostly high density/HDL or low density/LDL lipoprotein. Cholesterol is distributed by lipoprotein particles to body tissues and into the liver. Cholesterol transported in LDL complex is "bad" cholesterol because LDL also transports it into cells that line the inside of vessels by LDL. In contrast, cholesterol transported in HDL complex is referred to "good cholesterol" because HDL secretes cholesterol from cells and move it to the liver for partial elimination from body (Sherwood, 2003).

Diet containing *Gracilaria* sp. waste lowered cholesterol levels, so the amount of cholesterol transported by LDL to tissues also decreased. Bile salts directly stimulate the secretory cells of the liver to produce bile salts. Liver requires cholesterol to produce bile salt, when cholesterol is inadequate, liver will send messages to brain that responds by sending signals to HDL in liver to take the unused cholesterol (such as LDL), sending it through blood vessel tissue to liver, then using itin metabolic process (Lutfiana, 2006). Therefore, the higher HDL level, the lower LDL level.

Zahra et al. (2013) stated that the concentration of blood cholesterol affected levels of LDL and HDL. The higher cholesterol level, the higher HDL and LDL level, determined by genes, environment, and livestock welfare. Test results showed that blood cholesterol of local ducks decreased correlated with LDL and HDL levels.

Conclusions

Diet with 12.5%*Gracilaria* sp. waste plus multi-enzyme can improve blood lipid profiles of local ducks indicated by the decreased cholesterol, triglycerides, LDL, and the increased HDL. Further chemical test was important to figure out the diet response toward the quality of livestock product.

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