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The effects of native gedi leaves (*Abelmoschus manihot* L. Medik.) of Northern Sulawesi-Indonesia as a Source of Feedstuff on the Performance of Broilers

Jet Saartje Mandey[1,3*], Hendrawan Soetanto[2], Osfar Sjofjan[2], Bernat Tulung[3]

[1]*Post Graduate Student, Brawijaya University, Malang, The East Java, Indonesia*

[2]*Animal Nutrition Department, Animal Husbandry Faculty, Brawijaya University, Malang, The East Java, Indonesia*

[3]*Animal Husbandry Faculty, Sam Ratulangi University, Manado, The North Sulawesi, Indonesia*

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Abstract

In order to get information about growth and health promoter herbal plant as feedstuff in broiler chickens, gedi plant was investigated biology study. Gedi (*Abelmoschus manihot* L. Medik) is native plant and abundant in Northern Sulawesi-Indonesia. Utilization of gedi leaves as feedstuff in ration for broiler has not been conducted and reported in literatures. The objective of this research was to evaluate utilization of gedi leaves in ration on the parameters performance and histomorphological of villi ileum of broilers, as a possible feedstuff in enhancing and promoting growth of broiler chicks. Total of 100 unsexed day-old chicks (Cobb CP 707) were randomly allocated to four diet treatment groups, consisting of five replications in each treatment using five birds in each cell. Animals were fed commercial complete based diet. Dietary treatments were basal diet (R0), 95% basal diet + 5% gedi leaf meal (R1), 90% basal diet + 10% gedi leaf meal (R2), and 85% basal diet + 15% gedi leaf meal (R3). Treatments were administrated during 35 days. Feed and water were provided *ad libitum* throughout experiment. All diets were fed to birds as mash. Results showed that utilization of gedi leaves in ration affected normally on total blood cholesterol, edible giblet organs (liver and heart percentage) and histomorphological structures of villi ileum in broiler, but a highly significant increase feed conversion ratio and gizzard percentage was shown in treatment R3 (15%). Level of gedi leaves up to 15 percents in ration tended to reduce body weight gain, dressing percentage and abdominal fat in broiler. In conclusion, the result reported here indicating that addition of gedi leaf meal 5 to 15% to broiler diet enhanced the performance of broiler for functional food.

* **Corresponding Author:** Jet Saartje Mandey ✉ jetsm_fapet@yahoo.co.id

Introduction

Gedi is a native plant and abundant in Northern Sulawesi-Indonesia. Plant identification and moleculer characterization of morphological features of that species using DNA sequencing showed that gedi was species of *Abelmoschus manihot* (L.) Medik, tribe Malvaceae (Mandey 2013, unpublished). *Abelmoschus manihot* L. Medik is a plant with large annual erect hairy leaves, 1.2 – 1.8 m height throughout tropical region. In North Sulawesi island of Indonesia this plant, gedi leaves were used by local people to be the essential ingredient of the traditional porridge called “tinutuan” to give a special taste and viscousity. Its viscousity is associated with the content of gum (mucilage) containing polysaccharides and protein (Kiritikar and Basu, 1994 cited by Jain and Bari, 2010). Morphologically, gedi plants vary in the shape and color suggesting some genetic variation that may occur after a long period of adaptation.

Recently, gedi plants have been the subject of the number of experiments elucidating the other chemical compounds that may contribute to human health. Wang *et al.* (2012) reported that all of its flowers, leaves, stems and roots can be used as medicines, which were found to have anti-inflammatory, antibacterial and anticoagulant properties useful for treatment of chronic renal disease, mouth ulcers and burns. Its pharmacological action is caused by flavonoids, alkaloids, polysaccharides, and others, and the polysaccharide is viscous water-soluble macromolecule. Puel *et al* (2005) challenged the hypothesis that osteoporosis develops over the deficiency of estrogen by conducting an experiment using ovariectomy female wistar rat. In this experiment they found that feeding with high dose of gedi leaves prevented osteopenia, and the amount of calcium in leaves is very important (28.65 ± 0.37 mg/g of dry matter) as the protective effect in *A. manihot*. This finding may be associated with the report of Jain *et al* (2009) that found a significant amount of phytosterol (stigmasterol, γ-sitosterol) in the woody stem of gedi plant. Jain *et al* (2011) reported that extract of gedi leaves possess potential pharmacological active constituents responsible for inhibition of the analgesic effect. Gedi plant also contains isoquercitrin, hyperoside, hibifolin, quercetin-3’-o-glucoside, quercetin and isorhamnetin that can be associated with anticonsulvant and anti depressant-like activity after oral administration (Guo *et al*, 2011). In addition, this plant contains saponins, alkaloids, steroids, flavonoids and triterpenoids that are the main phytoconstituents (Todarwal *et al.*, 2011). The flower of this plant contains myricetin, cannabiscitrin, myricetin-3-o-beta-D-glucopyranoside, glycerolmonopalmitate, 2,4-dihydroxy benzoic acid, guanosine, adenosine, maleic acid, heptatriacontanoic acid, 1-triacontanol, tetracosane, beta-sitosterol, and beta-sitosterol-3-o-beta-D-glucoside (Lai *et al*, 2006). Sarwar *et al* (2011) stated that *A. manihot* had a profound anti-inflammatory and anti-diabetic effect. Wu *et al* (2007) reported that this plant had antiviral activity of hyperoside. Jain and Bari (2011) found that the woody stem had antimicrobial properties.

Research in Indonesia, Jeni Tresnabudi (1992) reported that gedi leaves (*Abelmoschus manihot* (L.) Medik, Malvaceae contained flavonoid compound, phenolic acid including pherural acid, siringic acid, and chlorogenic acid. Maryana Brotosudirdjo (1994) reported that gedi leaves contained flavonoid compound, steroid and triterpenoid compounds. Mamahit (2009), Mamahit and Soekamto (2010), and Mamahit (2011) reported that that gedi leaves of the North Sulawesi-Indonesia contained secondary metabolic β-sitosterol and heptadecanoic acid as the basic potential of drug development (Mandey 2013, unpublished) found that gedi leaves growing in Manado, North Sulawesi of Indonesia contained more steroid, flavonoid, alkaloid and saponin; and contained also high protein, crude fiber and calcium (29.2 – 37.0 mg/g of dry matter). Utilization of gedi leaves as a feedstuff in ration for broiler has not been conducted by researchers and reported in literatures. Therefore, the objective of this research was to evaluate utilization of gedi leaves in the feeding of broilers on the parameters performance and histomorphological of villi ileum of broilers, as a possible feedstuff in enhancing and promoting growth of broiler chicks.

Material and methods

Biological material

A total of 100 day-old broiler chicks (Cobb CP 707) were individually weighed (average 44.94 ± 1.98 g, coefficient of variance 4.40%) and randomly allocated to the four diet treatment groups, consisting of five replication in each treatment with five birds in each cell. At the end of the experimental period (35 days of age) five birds per treatment were slaughtered for carcass, blood and histomorphological analysis.

Experimental design and treatment

Experimental diets were formulated according NRC (1994). Animals were fed commercial complete based diet (BD) and gedi leaf meal (GLM). Dietary treatments were basal diet (control = R0), 95% basal diet + 5% gedi leaf meal (R1), 90% basal diet + 10% gedi leaf meal (R2), and 85% basal diet + 15% gedi leaf meal (R3). These treatments were administrated for a 35 days period. Feed and water were provided *ad libitum* throughout experiment. All diets were presented to the birds as mash. The feed composition and nutrient content were shown in Table 1, were analyzed using AOAC (1996) procedure. A continous light of 24 hours for the 5 weeks was applied. The chicks were weighed individually on days 1, 7, 14, 21, 28 and 35 per pen.

The following parameters were evaluated during the experimental period: the average body weight, body weight gains, feed intake and feed conversion ratio (FCR). The bird body weight and feed intake per pen were measured weekly. Feed conversion ratio was calculated on a pen weight basis. At 35 days of age, one representative bird from each pen was slaughtered and its carcass parameters including dressing percent without the edible giblets (liver, gizzard, heart), abdominal fat, and relative weight of liver, gizzard and heart were determined. Blood sample from one randomly selected birds per pen was collected by wing-vein puncture using sterilized 27 gauge needles and 3 ml syringes into test tubes treated with heparin for measurement of concentrations of blood total cholesterol. Sera were harvested from clotted blood by sentrifugation at 2000 g for 15 min. Serum samples were kept in 24°C until measuring cholesterol. Cholesterol was measured by enzymatic colorimetric test, CHOD-PAP method. At 6, 16 and 26 days of age, each bird received one dose of commercially newcastle disease virus (NDV) vaccine.

The gastrointestinal morphometric variable evaluated were villus height, villus width and crypt depth from ileum. A 5 cm segment of the midpoint of the ileum was dissected. Samples of the ileum were spread on polystyrene plates and fixed in 10% buffered formalin. The intestinal wall was precisely cut. Sections of 5-8 mm thickness were taken from each sample and fixed in 10% formalin solution and four slides were prepared from each sample. They were stained with hematoxylin and eosin and embedded in parrafin. Villus height, width and crypt depth were evaluated under a light microscope (Sakamoto *et al*, 2000). Data acquisitions were performed with Olympus BX51TF and SZX1 microscope using an ocular micrometer. At the end of the trial, the pH of ileum content was measured.

Statistical analysis

The value obtained were expressed as mean ± SD. In order to analyze the relation between intergroup mean differences, a completely randomized design (CRD) was employed in one-way analysis of variance, and significant differences compared by Duncan's multiple range tests. All of statement of differences were performed at significance levels of 1% and 5% (Snedecor and Cochran, 1962). Software package Genstat 12.2 was used for statistical calculation.

Results

Growth performance and carcass traits

Daily feed intake, body weight gain and feed conversion ratio during the entire trial period are shown in Table 2. It was observed that the feed intake of the animals fed gedi leaf highly significant decrease ($P<0.01$) compared to the control group. The body weight gain of the birds fed with gedi leaf was significantly lower (P<0.01) than that of non gedi leaf (control diet), and there was highly significant increase (P<0.01) in feed conversion ratio. FCR between chicks fed ration containing 5% and 10% gedi leaf and those fed ration as the control diet was no significant differences. However, ration containing 15% recorded a highly significant increase than that of 0%, 5% and 10%.

Table 1. Diet Formulation and Chemical Composition (*as fed*).

Ingredients (%)	BD[a]	GLM[b]	R0 (0% GLM)	R1 (5% GLM)	R2 (10% GLM)	R3 (15% GLM)
Basal Diet (%)			100	95	90	85
Gedi Leaf Meal			0	5	10	15
Calculated Analysis:						
Dry Matter (%)	93.38	81.72	93.38	92.80	92.21	91.63
Crude Protein (%)	22.34	20.18	22.34	22.23	22.12	22.02
Crude Fiber (%)	4.66	17.53	4.66	5.30	5.94	6.59
Fat (%)	3.15	1.06	3.15	3.05	2. 4	2.84
Nitrogen-Free Extract (%)	57.26	31.17	57.26	55.96	54.65	53.35
Ca (%)	1.28	3.29	1.28	1.38	1.48	1.58
P (%)	0.71	0.39	0.71	0.69	0.68	0.66
Methionine (mg/g)	17	16	17.0	17.0	16.9	16.9
Lysine (mg/g)	47	425	47.0	65.9	84.8	103.7
GE (kal/g)	3685	3419	3685	3671	3658	3645
Fiber Component:						
NDF (%)	68.67	20.78	68.67	66.28	63.88	61.49
ADF (%)	21.02	18.44	21.02	20.89	20.76	20.63
Hemicellulose (%)	47.65	2.34	47.65	45.38	43.12	40.85
Cellulose (%)	6.13	11.39	6.13	6.393	6.656	6.919
Lignin (%)	14.78	5.88	14.78	14.34	13.89	13.45
Silica (%)	0.09	1.5	0.09	0.14	0.20	0.5

Notes: [a] BD = Basal diet is a commercial ration with this composition: corn, rice bran, fish meal, soybean cake, coconut cake, meat and bone meal, oat, peanut cake, canola, leaf meal, vitamin, calcium, phosphate, and trace mineral; [b]GLM = gedi leaf meal; R0, R1, R2, R3 = dietary treatments.

Furthermore, the treatments affected significantly (P<0.05) the carcass percentage of broiler. Carcass percentage fed ration of R1, R2 and R3 treatments decreased significantly (P<0.05) compared with that fed ration of R0 treatment. However, carcass percentages among treatments of R1, R2 and R3 were not significantly different (P>0.05). This research revealed that utilization of gedi leaves of 5%, 10%, and 15% in ration decreased carcass percentage of broiler ranging from 5.70% to 6.55 % compared with R0 as control ration (Table 2). Results showed no significant differences (P>0.05) among treatments liver percentage and heart percentage, however, utilization of gedi leaves of those percentages in ration affected significantly (P<0.05) the variable gizzard percentage.

The treatments affected significantly (P<0.01) the abdominal fat percentage of broiler. Abdominal fat percentage of broiler fed ration of R2 and R3 treatments decreased significantly (P<0.01) compared with those fed ration of R1 and R0 treatments. In addition, the abdominal fat percentage fed ration of R1 decreased also significantly (P<0.01) compared with that fed ration of R0 treatment (Table 2). This study indicated that utilization of gedi leaves of 5, 10, and 15 percents in ration decreased the abdominal fat percentage of broiler ranging from

58.02 to 83.33 percents compared with R0 as control ration.

Table 2. Growth performance and carcass traits during the entire trial period for the broiler treatment groups.

Measurement	Diet				*Pvalue*
	R0	R1	R2	R3	
ATFI (g/b)	2708±35.33	2362±50.82	2044±73.46	1869±55.51	p<0.001
AFI (g/b/d)	77.38±1.01[d]	67.48±1.46[c]	58.41±2.13[b]	53.40 ± 1.59[a]	p<0.01
BW (g/b)	1754.4 ± 82.33	1354.5 ± 39.99	1067.4 ± 49.90	935.4 ± 52.40	
BWG (g/b)	1708.36±44.48[d]	1308.28±40.32[c]	1020.56±49.97[b]	888.84±52.82[a]	p<0.01
FCR (NU)	1.56 ± 0.05[a]	1.86 ± 0.13[ab]	2.01 ± 0.10[bc]	2.29 ± 0.41[c]	p<0.01
Dressing %	72.31 ± 0.65[b]	68.76 ± 3,64[a]	68.19 ± 2.10[a]	67.57 ± 2.61[a]	p<0.036
Abdominal Fat %	1.618 ± 0.32[c]	0.682 ± 0.16[b]	0.302 ± 0.05[a]	0.270 ± 0.09[a]	p<0.01
Liver %	2.202 ± 0.23	2.198 ± 0.30	2.162 ± 0.21	1.878 ± 0.17	p>0.05
Gizzard %	1.086 ± 0.20[a]	1.168 ± 0.18[a]	1.056 ± 0.09[a]	1.500 ± 0.26[b]	p<0.008
Heart %	0.470 ± 0.08	0.522 ± 0.14	0.418 ± 0.09	0.490 ± 0.06	p>0.05

ATFI = average total feed intake, AFI = average feed intake, BW = body weight, BWG = body weight gain, FCR = feed conversion ratio, g/b = grams per bird, g/b/d = grams per bird per day, NU = no unit; *Pvalue* = probability value; a, b, c = means followed by different letters within rows are different by Duncan's multiple range test in 0.05 significance level.

Total blood cholesterol, pH digesta and histomorphologica ileum

Results showed no significant differences (P>0.05) among treatments in total cholesterol, villi height, villi width, and crypt depth (Table 3). However, utilization of gedi leaves of those percentages in ration affected significantly (P<0.05) the variable of digesta pH. Digesta pH of broiler fed ration of R1, R2 and R3 treatments increased significantly (P<0.01) compared with those fed ration of R0 treatment as the control. In addition, gizzard percentage of broiler fed using R3 treatment increased significantly (P<0.01) compared with those fed ration of R0, R1 and R2 treatments.

Table 3. Total Blood Cholesterol, pH Digesta and Histomorphological Ileum of Broiler at 35 days of age fed different levels of GLM.

Measurement	Treatments				*Pvalue*
	R0 (0% GLM)	R1(5% GLM)	R2 (10% GLM)	R3 (15% GLM)	
Total Cholesterol (mg/dl)	122.6±10.88	110.4±14.08	106.0±9.13	110.4±2.97	p>0.05
pH Digesta	6.24 ± 0.59[a]	6.82 ± 0.27[b]	6.84 ± 0.19[b]	6.80 ± 0.07[b]	p<0.038
Villi Height (mm)	0.928 ± 0.15	0.973 ± 0.07	1.011 ± 0.09	0.986 ± 0.25	p>0.05
Villi Width (mm)	0.275 ± 0.07	0.195 ± 0.02	0.226 ± 0.17	0.196 ± 0.08	p>0.05
Crypt Depth (mm)	0.291 ± 0.13	0.213 ± 0.05	0.170 ± 0.04	0.191 ± 0.05	p>0.05

Notes: GLM = gedi leaf meal; mm = millimeter; Pvalue = probability value; a, b = means followed by different letters within rows are different by Duncan's multiple range test in 0.05 significance level.

The increase of gizzard percentage in broiler up to 37.61 percents could be due to higher crude fiber percentage in ration of R3 treatment up to 6.69 percent compared with that in ration of R0 treatment of 4.66 percent (Table 1).

The utilization of gedi leaves in ration did not affect (P>0,05) the histological figures of broiler ileum. Histological structure of broiler ileum fed ration R0 as control and ration R1, R2 and R3 showed the same structure of villi of broiler ileum (Figure 1). This condition might be due to lower feed intake in ration of R1, R2 and ration R3 causing lower supplementation of the gedi leaf in this rations.

Discussion

In general, there were significant reduced in feed intake, body weight gain and feed to gain ratio were observed in broilers fed the different experimental diets. Although it was expected that supplementing the dietary herbs would stimulate the growth performance of broilers (Cross *et al*, 2007), research on herbs yielded contradicting results. The research of the present study are in agreement with previous observations that indicated herbs affected to reduce body weight gain, feed intake or feed efficiency in broilers (Hernandez *et al*, 2004 cited by Stef and Gergen, 2012; Cross *et al*, 2007). Decrease of feed consumption might be due to the physical form of gedi leaves used in this study. The gedi leaves contained high mucilage with high viscousity. Mucilage is a type of soluble fiber of viscous nature. This high mucilage caused the difficulty of chicks to feed ration containing high gum. Consequently, it reduced feed consumption of chicks. Feed intake can be influenced by a large number of factors. Selection of food depends on visual appearance, temperature, viscosity, saliva production, nutritive value of feed, toxicity of feed components, particle size and social interaction (Blair, 2008 cited by Hippenstiel *et al*, 2011). Iji (1999) reported that the well known negative effects of non-starch polysaccharides on poultry productivity derive mainly from increases in the viscousity of digesta of birds fed the diets containing this materials. However, it had not been conclusively established how viscousity reduces digestive and absorptive functions in broiler chickens. Decrease of broiler average daily weight gain fed ration containing higher gedi leaves might be caused by lower fed consumption rate. Just like feed intake, body weight gain also depends on several factors like feeding system and diet attributes. Several studies confirmed the positive influence of herbs on BW gain. However, negative impacts on BW gain have also been reported. Decreasing daily weight gain of chicks occurred might also be due to low digestibility of ration fed to the animals (Mandey 2013, unpublished). Nguyen and Nguyen (2008) reported that increasing levels of NDF in the diets of growing crossbred rabbits was decrease daily weight gain and nutrient digestibility. Gidenne *et al.* (1998) and Perez *et al.* (1996) cited by Nguyen and Nguyen (2008) stated that an increase of fiber leads to decrease of retention time and an increase of caecotrophe production because of increasing bacterial fibrolytic activity which in turn results in a reduction of digestibility of diets. The feed conversion ratio (FCR) describes the relation of feed intake and BW gain. More precisely, it is the animal's overall efficiency in converting feed mass into body mass over a specific period of time (Hippenstiel *et al*, 2011). Kheiri and Rahmani (2006) reported that the circumtances of the poultry intestine is almost acidic than alkaline, and in this case high levels of Ca might increase the intestinal pH and consequently increase the digestion and absorption of the nutrients. As some have suggested removal Ca and P might influence the broiler performance. Iji (1999) reported that there was a dearth of research on the effect on nutrient transport in the gastrointestinal tract of poultry. Reports on depressed absorption of nutrients, including amino acids, are more common, such as this might be caused by an increase in digesta viscosity and a reduction in the movement of nutrients through a dense of unstrirred water layer rather than changes in the transport capacity of the mucosal membranes (Tulung *et al*, 1987). Pereira *et al* (2001) cited by Khatun *et al* (2010) reported that consumption of a diet high in soluble fiber has been suggested as a strategy to reduce the risk factors for development of obesity through the regulation of satiety and energy intake. Moreover, viscous water-soluble dietary fibers have the effects of hampering the diffusion of glucose and postponing the absorption and digestion of carbohydrates, thus resulting in lower postprandial blood glucose (Yokoyama *et al*, 1997, cited by Khatun *et al*, 2010).

Carcass weight and dressing percentage were significantly lower compared to the control, however between gedi leaf treatment had no different. Feeding broilers of gedi leaf 5% - 15% did not alter the percentage of edible giblet liver and heart. It could be attributed to gedi leaf effect on birds carcass and edible giblets organ that may be used as an altenative to feedstuff for improving their performance.

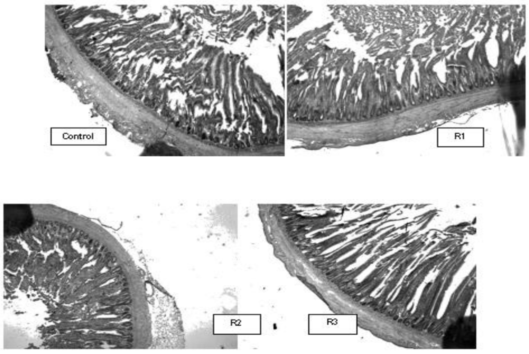

Fig. 1. Histological figure of broiler ileum for different treatment groups.

A normal cholesterol level in broiler ranged from 125 to 200 mg/dl, indicating that total cholesterol levels of broiler for all treatments in this study were below the normal standard. Those low total cholesterol might be due to high crude fiber levels ranging from 4.66 to 6.59 percents in rations. Maximum crude fiber content standard in ration of broiler was 5 percents (Leeson and Summers, 2001). McDonald *et al* (1995) and McDonald *et al* (2010) stated that high crude fiber in ration of animals might increase digest velocity in the intestine causing decrease of nutrient absorption and fat metabolic formation. On the other hand, the soluble fiber prevents the intestinal absorption of cholesterol produced by the bile for the digestion of feed. Soluble fiber forms a gel which traps that cholesterol that is expelled to the outside without passing into the bloodstream.

Inhibition of lipid oxidation was a chemical reaction mechanism related to the antioxidant function of the flavonoid and sterol (Fraga, 2010). Stigmasterol was a plant sterol group or fitosterol, and chemically equal to animal cholesterol. Result of this study indicated that stigmasterol might be useful to inhibit cholesterol absorption and decreased serum cholesterol level by competition of intestinal absorption. Stigmasterol was also potential as antioxidant, hypoglycemic and property to inhibit thyroid. Normally, fitosterol was digested in heart tract, either it is alone or combined with beta-sitosterol might reduce blood cholesterol level, so it was usually used in the treatment of hypercholesterolemia. Beta-cholesterol inhibit cholesterol absorption in the intestinal tract. Sterol was absorbed in intestine, carried by lipoprotein, and entered into cell membrane. Fitosterol and fitostanol inhibit digestion and cholesterol transportation, reduce LDL level and total cholesterol serum. Structure of beta-sitosterol was equal to cholesterol. Beta-sitosterol played role for cholesterol digestion and transportation to produce micelle in the intestinal lumen. This condition caused low cholesterol absorption in body metabolism.

The gedi leaf had no effect on villus height, villus width and crypt depth, however, tend to increasing the villus height. It may support the idea that the active principles of herbs act as a digestibility enhancer, stimulating the secretion of endogenous digestive enzymes. The lack effect of the supplements may be related to the environment conditions (Hernandez *et al*, 2004 cited by Stef and Gergen, 2012). Iji *et al* (2001) reported that increase nutritive value of ration containing low calorie by supplementation of microbial enzyme did not relate with the structure of villi. Structure of intestinal mucosa and enzyme function were not affected by microbial enzyme supplement. Research using commercial polysaccharides non-starch supplement caused change of ileum structure to enlarge compared with structure of jejunum. Changes of structure and function of intestine might be temporary or permanent.

Table 2 shows that the body weight, carcass percentage and abdominal fat were significantly decreased by gedi leaf compared to the control. However, between the level of gedi leaf 5%, 10% and 15% had no significant difference. There were no significant different FCR in treatment R0, R1 and R2. Therefore, it may be said that the gedi leaf has a advantage in the performance were observed between treatments.

Conclusion

Our data indicated that utilization of gedi leaves in ration affected normally on the feed conversion ratio, internal organ weight, and histomorphological structures of ileum villi in broiler. However, tended to reduce percentage of carcass and abdominal fat in broiler. Consequently, it can be suggested that gedi leaves can be used particularly in diets of chick birds where digestion problems at growing lead to scouring, and the reducing of abdominal fat should be taken into account for carcass quality as a functional food and deserves further study.

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