

## Cholesterol, Glucose and Blood Cells Count of Rabbit Doe Fed *Katuk* (*Sauropus androgynus* L. Merr) Leaf Meal as Supplementation

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**Abstractl.** This study was aimed to determine the ability of *katuk* that can affected cholesterol, glucose, erythrocyte, leukocyte, and thrombocyte during 3 weeks treatment. Twenty four does were raised from 6–12 month old, divided into three blocks, four treatment diets, and two replications. The treatments were P0= control diet, P1= 99% control diet + 1% *katuk* leaf meal, P2= 98% control diet + 2% *katuk* leaf meal, and P3= 97% control diet + 3% *katuk* leaf meal. The variables observed were cholesterol, glucose, erythrocyte, leukocyte, and thrombocyte. Cholesterol and glucode were determined with colorimetri methode with GOD-PAP, erythrocyte, leukocyte, and trombocyte with haemocytometer. Data were analyzed using analysis of variance (anova), followed by Duncan's Multiple Test Range. The results showed that addition of *katuk* leaf meal can decreased cholesterol (50.67–48.34 mg/dl) and glucose (119–115 mg/dl) and increased erythrocyte ( $6.17\text{--}6.25 \times 10^6/\text{mm}^3$ ) during 3 weeks experiment, whereas leukocyte, and thrombocyte not affected by *katuk* leaf meal. It is concluded that addition *katuk* leaf meal can decreased cholesterol and glucose and increased erythrocyte, whereas leukocyte and thrombocyte not affected by *katuk* leaf meal.

**Key words:** *Katuk* leaf meal, cholesterol, glucose, blood cell

**Abstrak.** Penelitian bertujuan untuk mengetahui pengaruh pemberian tepung daun *katuk* terhadap kadar kolesterol darah, glukosa darah, eritrosit, leukosit, dan trombosit kelinci betina. Dua puluh empat kelinci betina umur 6–12 bulan dibagi kedalam tiga kelompok dengan empat perlakuan pakan masing-masing dua ulangan. Perlakuan pakan yang diberikan adalah P0= pakan kontrol, P1= 99% pakan kontrol + 1% tepung daun *katuk*, P2= 98% pakan kontrol + 2% tepung daun *katuk*, dan P3= 97% pakan kontrol + 3% tepung daun *katuk*. Variabel yang diamati adalah kolesterol darah, glukosa darah, eritrosit, leukosit dan trombosit setelah 3 minggu perlakuan. Kadar kolesterol dan glukosa diukur dengan metode kolorimetri menggunakan GOD-PAP, jumlah eritrosit, leukosit, dan trombosit dengan haemocytometer. Data yang diperoleh dianalisis menggunakan analisis variansi, dilanjutkan dengan Uji Jarak Berganda Duncan's. Hasil penelitian menunjukkan bahwa penambahan daun *katuk* dapat menurunkan kadar kolesterol (50,67–48,34 mg/dl) dan glukosa darah kelinci betina (119-115 mg/dl) dan meningkatkan eritrosit ( $5,93\text{--}6,25 \times 10^6/\text{mm}^3$ ). Sedangkan leukosit dan trombosit tidak terpengaruh oleh penambahan tepung daun *katuk*. Kesimpulan penelitian adalah penambahan tepung daun *katuk* pada pakan dapat menurunkan kadar kolesterol dan glukosa darah kelinci betina, dan meningkatkan jumlah eritrosit tetapi tidak mempengaruhi jumlah leukosit dan trombosit.

**Kata kunci:** Tepung daun *katuk*, kolesterol, glukosa, sel darah

### Introduction

*Katuk* (*Sauropus androgynus* L. Merr) is one of the plants are easy to grow and usually found in tropical regions like Malaysia, Thailand, and Indonesia. According to Malik (1997) in addition to increase milk production *katuk* leaves also contain chemical compounds that can be used as the basis of drugs such as tannins (catechin), flavonoids, alkaloids, triterpenes, organic acids, astiri oils, saponins,

sterols, amino acids, proteins, carbohydrates, vitamins and minerals. Padmavathi and Rao (1990) also found the chemical compounds alkaloids papaverin (PPV) in *katuk* leaves suspected to have physiological effects in the body. Among the 11 vegetables from Indonesia (*katuk*, kenikir, kedondong china, antanan, kemangi, beluntas, mangkokan, ginseng leaf, pohpohan, kecombrang, and krokot) leaves *katuk* had the highest flavonoid content is 143

mg/100 g fresh weight or 832 mg/100 g dry weight (Andarwulan et al., 2010). The benefits of flavonoids is to protect the cell structure, increase the effectiveness of vitamin C, anti-inflammatory, allergy, hepatoprotective, antithrombotic, antiviral and anticarcinogenic, prevent bone loss and as a natural antibiotic (Middleton et al., 2000). Subekti (2007) states that *katuk* leaf meal in quail diet can produce low cholesterol yolk and meat product. This study aims to determine the effect of *katuk* (*Sauropus androgynus* L. Merr) leaf meal on levels of blood cholesterol, blood glucose, erythrocytes, leukocytes, and platelets in the rabbit doe.

## Materials and Methods

Twenty four rabbits doe were used in this study is a kind of local doe age range 6-12 were placed on battery cage systems (each containing one doe). *Katuk* leaf meal obtained from the leaves of plants older than 2 years, taken from the center of the plant washed and then dried in the sun until wilted. Drying was continued by using the oven on temperature

60°C for 24 hours. *Katuk* leaves have tried squeezing, if it is easy to break then grind to get *katuk* leaf meal to be mixed in the diet control. Diet given during this study were divided into 4 treatment P0: control diet; P1: 99% control diet + 1% *katuk* leaf meal; P2: 98% control diet + 2% *katuk* leaf meal; P3: 97% control diet + 3% *katuk* leaf meal.

Feeding is done 2 times that in the morning (7:00 to 8:00 am) given concentrates and evening (15:00 to 16:00 pm) was given in the form of green cabbage leaves. Feeding is provided on a limited basis to the needs of rabbit, forage 700 g and concentrate 170 g. Rabbits will be given diet treated for 3 weeks. The composition of diet ingredients and diet nutrients content of treatment can be seen in Table 1.

Blood sampling was done early in the day before were fed, in the final week of treatment. Blood was drawn section of existing veins in the ear (*Auricularis lateralis*), and then inserted in EDTA vacutainer tubes so that blood does not clot. Some blood samples are used for the purposes of calculating the number of blood

Table 1. The composition of ingredients and nutrients content of the treatment diet

Ingredients (%)	Treatments			
	P0	P1	P2	P3
Cabbage leaf	60	60	60	60
Pollard	26	25.5	25	24.5
Corn meal	5	5	5	5
Soybean meal	5	4.5	4	3.5
Molases	2	2	2	2
Mineral	1	1	1	1
Salt	1	1	1	1
<i>Katuk</i> leaf meal	0	1	2	3
Total	100	100	100	100
Nutrient content				
Dry Matter (%)	84.89	84.93	84.98	85.02
Crude Protein (%)	17.85	17.82	17.79	17.76
Crude Fiber (%)	12.79	12.81	12.84	12.86
Crude Fat (%)	3.37	3.39	3.41	3.43
Ash (%)	14.00	14.05	14.08	14.12
Gross Energi (kkal/kg)	2665.85	2654.12	2636.44	2632.81

Based on laboratory analyzes of Nutrition Food Animal Husbandry, Brawijaya University

P0: control diet; P1: 99% control diet + 1% *katuk* leaf meal; P2: 98% control diet + 2% *katuk* leaf meal; P3: 97% control diet + 3% *katuk* leaf meal.

cells (blood cell count) erythrocytes, leukocytes, and platelets last remaining be sentrifuge to get sediment and serum. Serum used for analysis of total cholesterol and fasting glucose.

**Cholesterol and fasting glucose.** Cholesterols and glucose were determined with CHOD-PAP-Method by Human, there is enzymatic Colorimetric Test. Cholesterol with Lipid Clearing Factor whereas glucose with glucose oxidase and peroxidase. Samples/standards taken as much as 10  $\mu$ L to in the cuvette. Furthermore, the enzyme reagent added of 1000  $\mu$ L and shaken, the mixture was incubated at room temperature for 10 minutes, after that absorbance sample/standard was measured against reagent blank, with a wavelength of 500 nm. Hitachi spectrophotometer used brand models U-2001 UV/V is pectrofotometre, lamp: Tungsten Iodide, detector: Silicon photodiode.

**Erythrocytes, leukocytes, and platelets usehaemocytometer method.** Suck blood from the tube using a pipette to strip 0.5, then suck Hayyem solution to strip 101 (for erythrocyte), Turk solution to strip 11 (for leukocyte), Ress Ecker solution to strip 101 (for Platelets) using a pipette containing a blood. Grasp both ends of the pipette with thumb and middle finger and shake pipette to form like a figure eight, so the solution and blood mixed. Take a room count with cover glass then drops 1 drop of blood from pipette and then cover with a cover glass. Then put it under a microscope and count the number of blood cells contained in small boxes. Red blood cells counted in five squares

are four corner boxes and a central box. The results of the final calculation of the total number of red blood cells from five squares are multiplied by 10,000 per  $\text{mm}^3$ . Whereas white blood cell counted in four boxes in the corner, the total number of white blood cells multiplied by 50. Platelets counted in big square in the middle, the total of platelets multiplied by 2000.

Observed variables blood cholesterol levels, blood glucose, erythrocytes, leukocytes and platelets after 3 weeks of treatment. The research design using a randomized block design (RBD) with 3 groups based on body weight, 4 diet treatments and 2 replications. Data were analyzed using analysis of variance (anova), followed by Duncan's Multiple Test Range (Steel and Torrie, 1993).

## Results and Discussion

### Effect of Treatment of Blood Cholesterol

Table 2 shows that blood cholesterol treated with the addition of leaf *katuk* lower compared with the control rabbits. Respectively P2 and P3 (48, 34 mg/dl), P1 (50.67 mg/dl), whereas P0 control (51.17 mg/dl). Based on the statistical analysis of the diet treatment provides significant effect ( $P < 0.05$ ) compared with controls. The highest decrease in P3 and P2 while the P1 decrease was not significantly different from P0. Adding *katuk* leaf meal 1% (P1) in the diet did not show significant differences with the control allegedly due to the provision of as much as 1% have not been able to affect the mechanism of the synthesis of cholesterol in the serum of rabbits. The decline

Table 2. Effect of treatment on levels of total blood cholesterol, glucose, erythrocytes, leukocytes, platelets doe during the study

Variabels	Treatments			
	P0	P1	P2	P3
Cholesterol (mg/dl)	51.17±2.65 <sup>a</sup>	50.67±2.16 <sup>ab</sup>	48.34±2.58 <sup>b</sup>	48.34±1.36 <sup>b</sup>
Glucose (mg/dl)	126±5.55 <sup>a</sup>	119±6.03 <sup>b</sup>	117.67±6.35 <sup>b</sup>	115.67±5.16 <sup>b</sup>
Erythrocytes ( $10^6/\text{mm}^3$ )	5.93±0.28 <sup>ab</sup>	6.17±0.16 <sup>bc</sup>	6.25±0.14 <sup>c</sup>	5.85±0.36 <sup>a</sup>
Leukocytes ( $10^3/\text{mm}^3$ )	6±0.66	5.60±0.48	6.17±0.89	5.95±0.89
Plateletes ( $10^3/\text{mm}^3$ )	131.67±2.58	131.5±1.87	132.5±1.87	131.34±1.50

Values bearing different superscript on the same row differ significantly ( $P < 0.05$ ). P0: control diet; P1: 99% control diet + 1% *katuk* leaf meal; P2: 98% control diet + 2% *katuk* leaf meal; P3: 97% control diet + 3% *katuk* leaf meal.

seen in the provision of new P2 (2%) and P3 (3%), despite a decline, but the decline is still in normal as stated by Smith and Mangkoewidjojo (1988) that the normal rabbit cholesterol by 10-80 mg/dl, whereas according Malole and Pramono (1989) level for rabbit normal cholesterol 35-53 mg/dl.

Decrease cholesterol serum levels in the blood due to the addition of *katuk* leaves meal is influenced by various factors like the presence of sterol in *katuk* leaves are known as phytosterols and papaverin or the active compound that serves as papaverin (papverin like compound). Phytosterols contained in *katuk* leaves are in the form of stigmasterol, sitosterol and fukosterol with a total of 2433.4 mg per 100 g of dried weight (Subekti, 2007). Phytosterols inhibit cholesterol absorption from the intestine, increasing the excretion of bile salts, or prevent esterification of cholesterol in the intestinal mucosa. Phytosterols inhibit cholesterol synthesis by modifying the activity of the hepatic enzyme acetyl-CoA carboxylase and cholesterol 7-hydroxylase (Silalahi, 2006). In addition to the active ingredient phytosterol mechanisms other alleged influence on blood cholesterol levels are alkaloida papaverin (PPV). Suprayogi (2000) states that papaverin like compounds have activity in leaves *katuk* among others inhibit fat absorption. This inhibition occurs because of impaired synthesis of bile that reduced bile secretion resulting in decreased absorption of fat. As a result of fat absorption following main components such as cholesterol decreased.

#### Effect of Treatment of Blood Glucose

Table 2 shows that the blood glucose levels of rabbits fed *katuk* leaves lower compared with the control rabbits. P3 (115.67 mg/dl), P2 (117.67 mg/dl), P1 (119 mg/dl), and P0 (126 mg/dl). Based on the statistical analysis of the addition of leaf *katuk* meal in diet significantly different ( $P < 0.05$ ) compared with controls. The highest decrease in P3 as diet with the addition

of *katuk* leaves meal highest 3% followed by P2 and P1. Despite the blood glucose decrease in treated but is still in normal. Anonymous (2000) stated that normal blood glucose in rabbits is 75–150 mg/dl. Meanwhile, according to Keeble (2001) normal blood glucose levels in rabbits ranged from 4.2 to 10.4 mmol/L (76.36 to 189.09 mg/dl). Liver function as a buffer system of blood glucose is essential. Blood glucose levels rising to very high concentrations after a meal accompanied by increased insulin secretion. As many as two-thirds of the glucose is absorbed by the intestine are stored in the liver in the form of glycogen. Over the next few hours when the concentration of blood glucose and insulin secretion rate is reduced, then the liver will release glucose back into the blood. Nugroho (2006) states that the mechanism of increased blood glucose levels regulated by the hormone glucagon from alpha cells, anterior pituitary growth hormone, epinephrine from the adrenal medulla and glucocorticoids from the adrenal cortex. *Katuk* leaves containing alkaloid compound or papaverin like compounds that inhibits gluconeogenesis and glycogenolysis in the liver so that the concentration entering the liver will increase without the accompaniment of an increase in the concentration of glucose leaving the liver, resulting in a surplus of glucose in the liver (Suprayogi, 2000). If the glucose in the liver is high then glukossa in blood will decrease.

#### Effect of Treatment of Total Erythrocyte

Table 2 shows that the amount of erythrocytes of rabbits fed *katuk* leaves with level 2% or at P2 increased and decreased the level of 3% or P3. Respectively P2 ( $6.25 \times 10^6 / \text{mm}^3$ ), P1 ( $6.17 \times 10^6 / \text{mm}^3$ ), P0 ( $5.93 \times 10^6 / \text{mm}^3$ ), and P3 ( $5.85 \times 10^6 / \text{mm}^3$ ). Based on the statistical analysis of the diet treatment significantly different ( $P < 0.05$ ) compared with controls. Highest increase in P2, while P1 and P3 not significantly different from P0. Despite an increase in red blood cells or erythrocytes

treated rabbits but the increase is still within the normal level as stated by Poljičak-Milas (2009) that the number of normal erythrocytes doe at  $4.89$  to  $6.85 \times 10^6/\text{mm}^3$ .

The main function of erythrocytes is to transport Hb (hemoglobin) containing oxygen from the lungs to the tissues. Papaverin in the blood can interact with hemoglobin with lower oxygen affinity and hemoglobin contained in erythrocytes (De Paula and Meirelles, 1992). The decrease in the oxygen affinity of Hb can stimulate the production of erythropoietin to form a new erythrocytes. Blood cell formation will continue until the oxygen demand in the tissues are met. Hb synthesized in erythrocytes and has the ability to bind to oxygen so that if the process of the formation of erythrocyte hemoglobin concentration continues to rise (Guyton and Hall, 2008). But not always increase papaverin in the blood causes an increase in the number of erythrocytes, this can be seen in P3 whose numbers began to decline, it is thought because of the time and materials required for the formation of erythrocytes is not proportional to the increasing number papaverin Hb binding. Despite the decline in the number of erythrocytes in P3 but this decline was normal in the number of rabbit does is  $4.89$  to  $6.85 \times 10^6/\text{mm}^3$  (Poljičak-Milas 2009) so as not to cause harm to the health of doe.

In addition topapaverin stimulates the increased number of erythrocytes the flavonoids also can make mature erythrocytes in the blood longer. Flavonoid capable of acting as an antioxidant, which serves rid of free radicals in the plasma membrane (Hafidz et al., 2009). Oxidative damage caused by free radicals that accumulate in the membrane components will affect the aging and destruction of erythrocytes is shorten life of erythrocytes. The presence of flavonoids in the *katuk* leaves able to prevent erythrocyte membrane damage caused by free radicals. Flavonoids affect the activity of some enzymes

attached to the plasma membrane alkaline phosphatase, carbonic anhydrase, and superoxide dismutase. The form of the cytosolic and extracellular superoxide dismutase (SOD) plays an important role as an antioxidant defense against free radicals by catalyzing superoxide radical ( $\text{O}_2^-$ ) to hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (Asgary et al., 2005).

#### **Effect of Treatment of Total Leukocytes**

Leukocytes is one of the active components of the blood cell and play a role in the body's defense system. Defense mechanism is to destroy the infectious agent through phagocytosis or by forming antibodies and sensitive lymphocytes (Bratawidjaja and Rengganis, 2010). In normal circumstances, peripheral blood of doe containing leukocytes about  $4.4$  to  $13.2 \times 10^3/\text{mm}^3$  (Poljičak-Milas, 2009). Table 2 shows the range of the number of leukocytes in the control rabbits and rabbits treated is still in the normal number. Providing *katuk* leaves meal in rabbits showed no significant difference ( $P > 0.05$ ) in the number of leukocytes rabbits. Successive from highest to lowest are P2 ( $6.17 \times 10^3/\text{mm}^3$ ), P0 ( $6.0 \times 10^3/\text{mm}^3$ ), P3 ( $5.95 \times 10^3/\text{mm}^3$ ) and P1 ( $5.6 \times 10^3/\text{mm}^3$ ). Number of white blood cells or leukocytes in general will be increased when the body is infected with microorganisms from outside the body.

Addition *katuk* leaves on diet indirectly also include flavonoids into the rabbit doe's body. Middleton et al. (2000) suggest that flavonoids may protect cell structure, increase the effectiveness of vitamin C, anti inflammatory, antibacterial, antiviral, and as a natural antibiotic. Flavonoids as natural antibiotics are sometimes caused by a reaction flavonoid itself and sometimes occurs because of interaction with other components in the body. Flavonoids can damage cell membranes and lyse bacteria so the flavonoid also can stimulate the body to secrete antibacterial liquid. In viruses, flavonoids function as a barrier membrane damage

outside of the body the virus so that the virus can not remove the protein in the cell to replicate DNA (Middleton et al., 2000). This ability is what can keep leukocyte production stable despite any foreign object into the body which are pathogenic and destructive.

#### Effect of Treatment of Number of Platelets

Table 2 shows that the number of platelets treated rabbits showed no significant difference compared with control rabbits ( $P > 0.05$ ). Successively from P0 control ( $131.67 \times 10^3/\text{mm}^3$ ), P1 ( $131.50 \times 10^3/\text{mm}^3$ ), P2 ( $132.50 \times 10^3/\text{mm}^3$ ), and P3 ( $131.34 \times 10^3/\text{mm}^3$ ). Platelets are small pieces that are not round or colored and shaped like a rod with a diameter of only 2–4 microns contained in the blood. Platelets are made in the liver, spleen and bone marrow since was a fetus. Platelets have multiple functions in the animal body, its main function is to prevent the occurrence of haemorrhagia (continuous bleeding) if injury (Guyton and Hall, 2008).

Referring to Andarwulan et al. (2010) which states flavonoid in dry weight of *katuk* leaf as much as 832 mg/100g, the consumption flavonoids from the leaves of *katuk* of P1 of 33.28 mg/day, P2 of 66.56 mg/day and at P3 of 99.84 mg/day. Guerrero (2004) states that the flavonoids in the blood can inhibit excessive platelet function by blocking the thromboxane A2 receptor so that the number of platelets in the blood is not excessive. Aggregation of platelets stimulated by thromboxane A2 produced with the help of the enzyme thromboxane synthetase. Aggregation platelets in the blood can cause excessive blood clotting or hypercoagulable. Hypercoagulation include increased platelet function and impaired fibrinolysis. In addition to blocking the thromboxane receptor, flavonoids in leaves *katuk* also prevent inflammation that damages blood vessels can be avoided. Decreased inflammation will prevent excessive platelet production quantities. This is why the addition

of *katuk* leaf meal can keep the platelet count remained stable between treatments.

## Conclusion

The addition of *katuk* leaf meal in doe's diet can decrease blood cholesterol and blood glucose, also increase the number of erythrocytes but doesn't affect the number of leukocytes and platelets in the blood.

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