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Reduction of Intracellular Lipid Accumulation, Serum Leptin and Cholesterol Levels in Broiler Fed Diet Supplemented with Powder Leaves of *Phyllanthus buxifolius*

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ABSTRACT

Phyllanthus buxifolius is an herbal plant, which is known to have antilipidemic and anticholesterolemic effects. The aim of this study was to explore the effects of leaf powder *P. buxifolius* supplementation in broiler feed on intracellular lipid accumulation, serum leptin and meat cholesterol levels. Sixty broiler chickens aged at 22 days were divided into two treatment groups. The first group was fed commercial feed without powdered leaves of *P. buxifolius* (control) and another group was fed commercial feed with 5% powdered leaves of *P. buxifolius*. The results showed that the accumulation of intracellular lipids, serum leptin levels, fat and cholesterol of meat and abdominal fat weight of chickens fed 5% powdered leaf *P. buxifolius* in feed were significantly lower than those fed the control diet. It can be concluded that administration of 5% powdered leaf *P. buxifolius* in broiler feed capable of lowering intracellular accumulation of lipids, serum leptin levels, fat and cholesterol of meat and abdominal fat weight.

Key words: *Phyllanthus,* lipid, leptin, cholesterol, broiler

INTRODUCTION

Public interest of broiler chicken meat that has low fat content and cholesterol levels in recent years tends to increase, especially for people with high risk of cardiovascular disorders and hypercholesterolemia. High levels of cholesterol in the blood can trigger the emergence of cardiovascular disease (Goldstein *et al*., 1973).

Broiler finisher feed generally contains a higher energy level than the starter period. Feeding broiler high fat diet can increase the fat tissue weight, total cholesterol, triglycerides and Low Density Lipoprotein (LDL) levels (Xia *et al*., 2010). Excess calories in the diet causes an increase in fat deposition and can stimulate increase secretion of leptin (Roth *et al*., 2008). More leptin synthesized may lead to increase the size of adiposities due to the accumulation of triglycerides (Taleb *et al*., 2007).

The continuous use of synthetic feed supplements stimulates growth in chickens, but the residue is difficult to decompose and may cause health problems to the consumers. Reported research on the feed supplemented with natural herbal showed reduction of abdominal fat levels without inhibiting the growth of broiler chickens. Provision of natural herbs that have potential

antilipidemic effect expected to also affect leptin levels and the accumulation of intracellular fat and lower adiposities in meat without influencing body weight. Natural antioxidants have been to offer vast array of health effects including lowering the cholesterol level (Nurulhuda *et al*., 2012).

Phyllanthus buxifolius (family; Euphorbiaceae) is a medicinal plant and has been widely used to treat various types of diseases by Indonesian people. *Phyllanthus* genus contains many medicinal secondary metabolites (Zhang *et al*., 2000). *P. buxifolius* leaves known to contain flavonoids, polyphenols (tannins), saponins, alkaloids, quinones, steroids and triterpenoids (Wardah *et al*., 2007). Flavonoids have the capacity as an antioxidant in the body of cattle (Gonzalez-Paramas *et al*., 2004) and suppressed the synthesis of fatty acids (Rodrigues *et al*., 2005) and adipogenesis in cells adiposities (Kuppusamy and Das, 1994). The present of polyphenols and flavonoids in chicken diet significantly reduced hyperlipidemia (Xia *et al*., 2010). Flavonoids and polyphenols also inhibit the enzyme activity of Glycerol 3-Phosphate Dehydrogenase (GPDH) in adiposities (Hsu and Yen, 2007). Meanwhile, saponins are known to inhibit the absorption of fat by the intestine and excreted through the feces (Dong *et al*., 2007). While tannins will bind to the protein in the body and lining of the intestinal wall so that protein digestion and absorption is inhibited and protein excreted in the stool increases (Matsui *et al*., 2006).

Ethanol extract of powdered leaves *P. buxifolius* of 240 and 320 mg fed daily to chickens was reported to reduce levels of fat and blood cholesterol without causing infection and inflammation (Wardah *et al*., 2007). However, the effect of powdered leaves of *P. buxifolius* in reducing levels of fat and meat cholesterol of broiler has not been revealed. This study aimed to examine the effect *P. buxifolius* leaf powder in feed on intracellular lipid accumulation, leptin and cholesterol levels of broiler meat.

MATERIALS AND METHODS

Preparation of *P. buxifolius* leaf powder: *P. buxifolius* was obtained from the garden of the Faculty of Veterinary Medicine Airlangga University and has been identified by the Center for Plant Conservation Purwodadi, Indonesia as *Phyllanthus buxifolius*. Leaves of *P. buxifolius* were separated from the stalk and seeds, dried room temperature for 5-7 days and in the oven with a temperature of 50°C for 3 h until the moisture content reached 10-12%. Leaves of *P. buxifolius* dry milled and sieved to 20 mesh size. The mixture then was made in the form of feed pellets and stored at room temperature until use.

Experimental design: Sixty Day Old broiler Chick (DOC) female (Cobb strain) was obtained from local hatchery (average weight of 43 g) and commercial feed was obtained from local factory (Japfa Comfeed). At the age of 1-21 days, the chickens were placed in cages equipped with electric brooder, feeder, drinker and lighting for 24 h. Chickens were fed a local commercial feed (Japfa Comfeed). The feed is given daily as much as 25 g at the age of 1-7 days, 56 g at the age of 8-14 days and 91 g at the age of 15-21 days and had free access to drinking water. At the age of 21 days chicken weighed and transferred into individual cages until the age of 42 days. A total of 30 chickens were given daily feed without powdered leaves of *P. buxifolius* (control group) and 30 chickens were given daily feed supplemented with 5% powdered leaves of *P. buxifolius* fed from the age of 22 to 42 days. At 22-28 old chickens old, each chicken fed daily 132 g, at 29-35 day old fed 169 g day^{-1}, at 36-42 days fed 200 g day^{-1} with free access to the drinking water. Vaccination programs were carried out in accordance with the provisions of local DOC factory producer.

Data collection: Feed consumption, body weight, carcass weight, accumulation of intracellular fat, serum leptin levels, fat and cholesterol of meat and abdominal fat weight of broiler chickens

were observed on day 7, 14 and 21 days after the treatment. Observations were made on 10 chickens of each treatment. Blood sampling performed at the jugular vein of chicken wings with sterile syringes and steraject (25G×1 ") and placed in blood collection tubes (13×75 mm) of 3-4 mL. Observation of intracellular accumulation of lipids, fats and cholesterol levels performed on tissue sections of chicken breast meat.

Flavonoid content in leaves of *P. buxifolius* was determined by the method of Switzerland Pharmacopoeia VII (Morais *et al*., 1999). Saponin was determined by color test reaction and thin layer chromatography (Harborne, 1996). Polyphenols (tannins) was determined by Folin-Ciocalteu method and UV-Vis spectrophotometer (Morais *et al*., 1999). Pectin, cellulose, hemicelluloses and lignin determined by the method developed by Gopal and Ranjhan (1980).

Analysis of serum leptin levels using a technique enzyme-linked immunosorbent assay (ELISA) and extra cellular fat accumulation with Oil Red-staining techniques on tissue O meat (Indra *et al*., 2010). Fat content in meat was analyzed by Soxhlet extraction method (AOAC, 1999). Meat cholesterol levels were analyzed by the method of Folch-Chod PAP spectrophotometer (Boehringer, 1993).

The study was complemented by research ethics (Ethical clearance) which has been approved by the Research Ethics Committee (Animal Care and Use Committee) Faculty of Veterinary Medicine University Press, No: 121-TO on April 29, 2011.

Statistical analysis: All data were analyzed using t-test. The data observed intracellular accumulation of fatty meat first performed using a percentage scoring, followed by t-test was conducted by SPSS 13 software for Windows.

RESULTS

Serum leptin levels: The present study showed that serum leptin levels in broiler chickens fed 5% powdered leaves of *P. buxifolius* for 7, 14 and 21 days was significantly ($p<0.05$) lower than serum leptin levels in control chickens group. The mean serum leptin levels of broiler chickens were presented in Fig. 1.

Feed consumption: The present study showed that feed consumption of broiler chickens fed powdered leaves of *P. buxifolius* for 7, 14 and 21 days did not differ significantly ($p>0.05$) compared with feed consumption of control group. Average feed consumption was presented in Fig. 2.

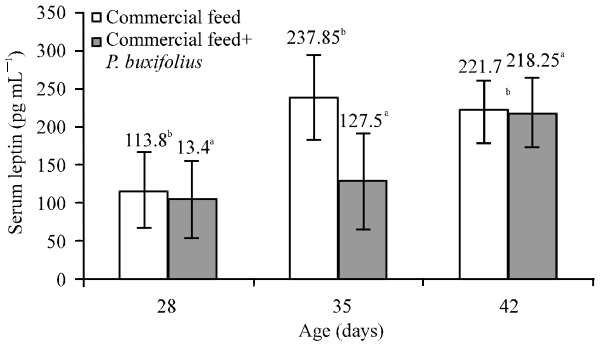

Fig. 1: Effect of *P. buxifolius* leaves on leptin serum levels in broiler chicken, Values represent the Mean±SD of 10 measurements, Values On each bar with different letters are significantly different at $p<0.05$

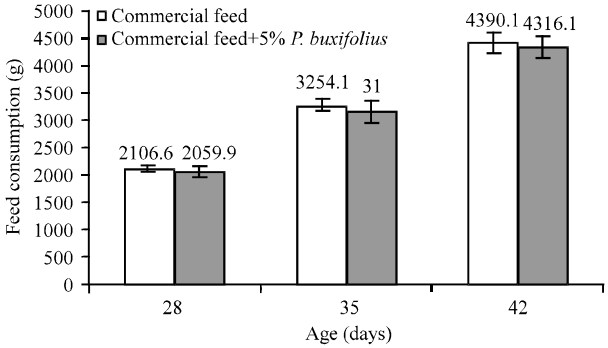

Fig. 2: Effect of *P. buxifolius* leaves on broiler chicken feed consumption, values represent the Mean±SD of 10 measurements

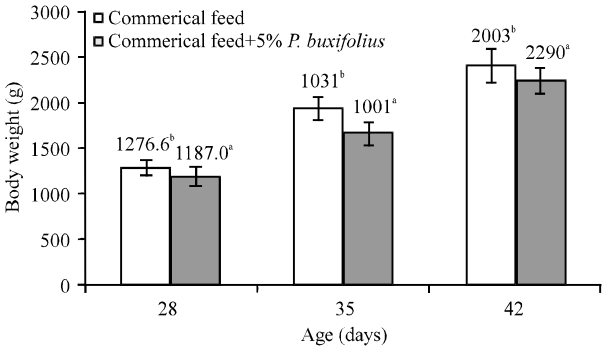

Fig. 3: Effect of *P. buxifolius* leaves on body weight broiler chicken at 42 days old, Values represent the Mean±SD of 10 measurements, Values on each bar with different letters are significantly different at $p<0.05$

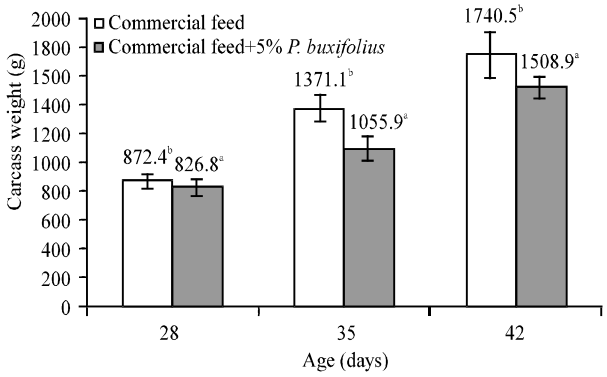

Fig. 4: Effect of *P. buxifolius* on carcasses weight of broiler chicken, Values represent the Mean±SD of ten measurements, Values on each bar with different letters are significantly different at $p<0.05$

Body and carcass weight: The present study showed that body (Fig. 3) and carcass (Fig. 4) weights of broiler chickens given 5% powdered leaves of *P. buxifolius* for 7, 14 and 21 days, respectively were significantly ($p<0.05$) lower than body and carcass weights of broiler chickens without supplementation of powdered leaves of *P. buxifolius* (control). The mean of carcass weight of broilers fed powdered leaves of *P. buxifolius* 66.0-69.62% while the mean of carcass weight of control group 68.4-72.4%.

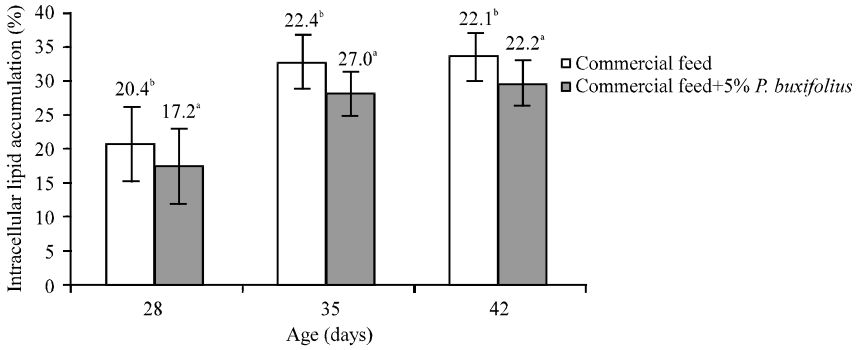

Fig. 5: Effect of *P. buxifolius* leaves on intracellular lipid accumulation in broiler chicken, Values represent the Mean±SD of ten measurements, Values on each bar with different letters are significantly different at p<0.05

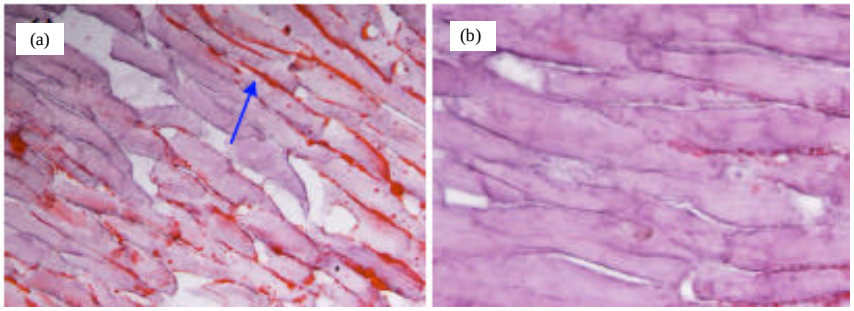

Fig. 6(a-b): (a) Section of meat tissue with lipid droplet staining with Oil Red O a network in broiler chicken were fed commercial feed without *P. buxifolius* leaves, (b) compared with the section meat tissue in broiler chicken that were fed commercial feed with 5% *P. buxifolius* (Bl.) leaves powder, blue arrow indicate the presence of lipid droplets that absorb color red with Oil Red O with higher color density(OD) (x400 magnification with photomicroscope OlympusCKX41), Scale bar: 0.55 μm on the objective lens

Intracellular lipid accumulation: The present of study showed (Fig. 5) that intracellular lipid accumulation of broiler chickens fed powdered leaves of *P. buxifolius* for 7, 14 and 21 days, respectively was significantly (p<0.05) lower than the intracellular lipid accumulation of control group. The section of broiler chicken tissue (Fig. 6) from control group were indicated the presence of lipid droplets that absorbed red with Oil Red O with higher color density than the section of broiler chicken tissue with fed 5% powdered leaves of *P. buxifolius*.

Abdominal fat weight: The present study showed (Fig. 7) that the weight of abdominal fat in chickens were fed 5% powdered leaves of *P. buxifolius* for 7, 14 and 21 days, respectively was significant (p<0.05) lower than the weight of abdominal fat in chickens of control group.

Meat fat and cholesterol levels: The present study showed that meat fat (Fig. 8) and cholesterol (Fig. 9) levels in broiler chickens fed 5% powdered leaves of *P. buxifolius* for 7, 14 and 21 days, respectively were significantly (p<0.05) lower than meat fat and cholesterol levels in broiler chicken control group.

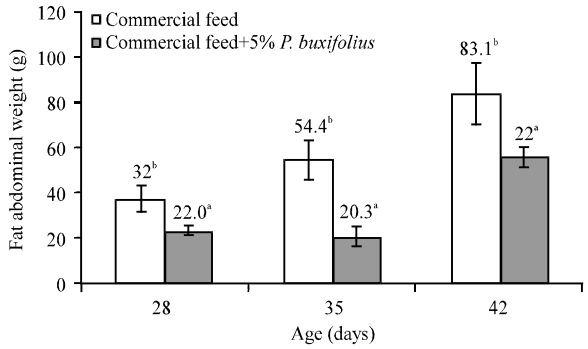

Fig. 7: Effect of *P. buxifolius* leaves on fat abdominal weight in broiler chicken, Values represent the Mean±SD of ten measurements, Values on each bar with different letters are significantly different at $p<0.05$

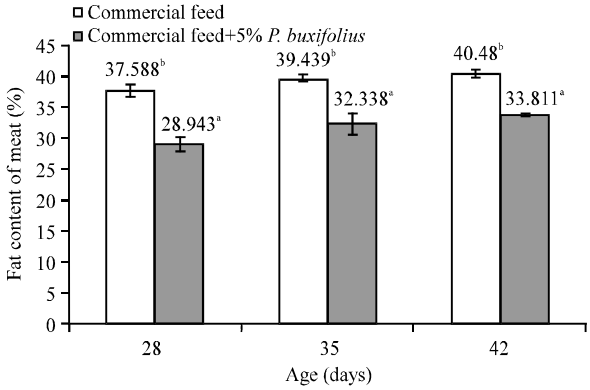

Fig. 8: Effect of *P. buxifolius* leaves on meat fat in broiler chicken, Values represent the Mean±SD of ten measurements, Values on each bar with different letters are significantly different at $p<0.05$

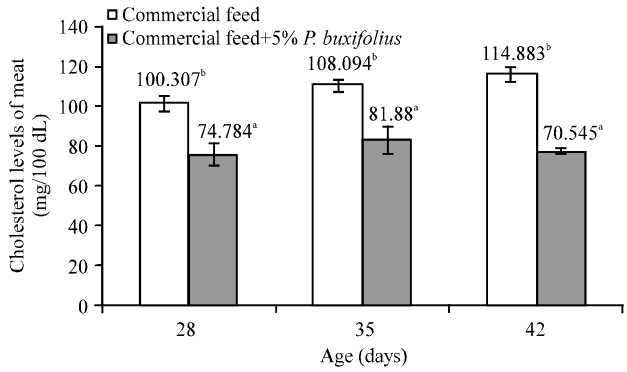

Fig. 9: Effect of *P. buxifolius* leaves on contain of meat cholesterol in broiler chicken, Values represent the Mean±SD of ten measurements, Values on each bar with different letters are significantly different at $p<0.05$

DISCUSSION

In vitro studies using various chemical assays have been used to assess the potential of peptides to act as cholesterol-reducing agents in order to minimize absorption of cholesterol in the human

body (Kongo-Dia-Moukala *et al.*, 2011). Polypeptide hormone leptin plays an important role in feed intake and energy metabolism (Ashwell *et al.*, 1999) and to maintain energy balance and body fat mass control with a feedback mechanism acting on the satiety center in the hypothalamus (Williams *et al.*, 2009). Leptin could play a role in enhancing the fertilization capacity oh human spermatozoa (Lampino and du Plessis, 2008). Leptin is also reported to have effects in peripheral tissues that are independent of its central effects on food intake and body weight (Nasri *et al.*, 2006) and might have a promoting effect on the carcinogenesis and progression prostate cancer (Kim *et al.*, 2008).

Excess energy consumption will affect the subsequent deposit of fat stimulates the secretion of leptin (Roth *et al.*, 2008). Increased levels of leptin would stimulate anorexigenic center in the hypothalamus to reduce production of neuropeptide Y (NPY) so that feed consumption decreases (Perusse *et al.*, 2001). Conversely, the chicken needs greater energy than the energy available in food causes reduced adipose tissue mass and stimulation of anorexigenic centers occurs which causes an increase in food consumption (Rodrigues *et al.*, 2005). The present study indicated that administration of 5% powdered leaves of *P. buxifolius* can reduced leptin levels, body weight and carcass weight, intracellular lipid accumulation, abdominal fat weight and fat and cholesterol level in broiler chickens. The present study also shown that hemicellulose, cellulose and pectin levels were increased with supplementation of 5% powdered leaves of *P. buxifolius*.

Fibers have an important role in inhibiting the absorption of fat and cholesterol in the digestive tract. Increase in crude fiber content in the feed can inhibit the process of lipogenesis (Murray *et al.*, 2003). The capacity of dietary fiber to lower serum cholesterol by hindering the digestion and absorption of dietary fat, modifying bile acid absorption and metabolism, forming a short chain fatty acid which can inhibit cholesterol and fatty acid synthesis in the liver and altering the concentration of insulin and hormones (Lecumberri *et al.*, 2007). Soluble fiber can slow the gastric emptying time and increase the transit time through the intestine, whereas insoluble fiber can shorten transit time and increase stool mass. Fiber can inhibit Nicotinamide Adenine Dinucleotide (NAD) and Nicotinamide Adenine Dinucleotide Phosphate (NADP) so that the process of lipogenesis inhibited (Murray *et al.*, 2003) Pectin in the gastrointestinal tract may reduce absorption of fat by binding to fatty acids. Thus, it is expected to cause the acceleration and excretion of fat through the feces and not synthesized in the process of lipogenesis.

Pectin can increase the viscosity and affect the process of digestion and absorption of food in the small intestine (Marounek *et al.*, 2007). The percentage of body fat has a positive correlation with serum leptin concentrations (Taleb *et al.*, 2007). The correlation of serum leptin levels with body fat mass increased with fat accumulation (Mohkam *et al.*, 2011). More leptin will be synthesized with an increase in the size of adiposities due to the accumulation of triglycerides (Wang *et al.*, 2010). *Phyllanthus* could inhibit fat accumulation in cells and tissues of meat and reduced urinary oxidative stress, inflammation and lowering repair of fat accumulation (Shen *et al.*, 2008). Aqueous extract of *Phyllanthus amarus* have reduced cholesterol concentration and low density cholesterol in albino rats (James *et al.*, 2010) and increase on the level of cGMP which might use as a sexual stimulant and a feedback effect on nitric oxide synthase which resulted to reduced concentration of nitric oxide in the plasma (Bankole *et al.*, 2011). Aqueous fruit extract *Phyllanthus emblica* has significant antidiabetic and hypotriglyceridemic activities (Qureshi *et al.*, 2009) and consumption of dried fruit of *P. emblica* have decreased total cholesterol, triglyceride and low density cholesterol (Ahmed *et al.*, 2010).

Secondary metabolic compounds can affect fat absorption and transport, as well as break down fat and cholesterol in the liver into bile salts, fats and cholesterol would subsequently excreted through the feces (Guyton and Hall, 2006).

Some species of *Phyllanthus* were known to contain many compounds of flavonoid and tannins which act as antioxidants (Wang, 1998). *Phyllanthus amarus* has antihyperlipidemic activity because they contain flavonoids, saponins and tannins (Adeneye *et al*., 2006; Umbare *et al*., 2009). The high amount of lignins, flavonoid present in *P. reticulatus* may be responsible for the hypocholesterolemic effect (Maruthappan and Shree, 2010). The results in present study showed that the powder of leaves of *P. buxifolius* contained polyphenols (tannins) 0.9 and 0.55% flavonoids per 100 mg and positive for saponins.

Cholesterol is a central molecule in animal physiology owing to its importance in the maintenance of cell structure, bile salt metabolism and steroid hormone synthesis (Saez *et al*., 2011). Polyphenol compound are believed to play a significant role in lowering plasma cholesterol by binding to bile acids which in turn increased faecal loss (Zunft *et al*., 2003). The possible cholesterol lowering effect was contributed by the presence of polyphenols such as phenolic acids, flavonoid, anthocyanidins and anthocyanin (Nurulhuda *et al*., 2012).

Flavonoids in the body have potential as inhibitors of differentiation of fat cells of the body so the body's fat cells inhibited maturation and decreased leptin synthesis (Roth *et al*., 2008). Some flavonoids have the potential to induce tissue lipolysis in adiposities (Kuppusamy and Das, 1994). Flavonoid also known to suppress the synthesis of fatty acid and triglyceride synthesis and inhibits cholesterogenesis in the heart that causes blood levels of triglycerides in the fall so that the accumulation of fat in the carcass and other body parts down (Santoso *et al*., 2000). Flavonoids and phenolic acids also act as natural antioxidants, potentially inhibiting the formation of fat from fat cells and affect the expression of leptin (Hsu and Yen, 2007). Natural antioxidants in the form of flavonoids and phenolic acids play a role in the inhibition of intracellular triglycerides and enzyme activity of glycerol 3-phosphate dehydrogenase (GPDH) in 3T3-L1 adiposities (Hsu and Yen, 2007). Licorice flavonoid have great potential as an agent for the treatment of anti-metabolic syndrome, oral administration of licorice flavonoid reduced the level of circulating glucose, insulin and leptin in mice (Ma *et al*., 2010).

Saponins plays a role in lowering cholesterol due to saponins can form insoluble complexes bond with cholesterol from food in the gut, so that cholesterol can not be reabsorbed by the intestine (Ueda, 2001; Dong *et al*., 2007). Saponins can combine with bile acids and cholesterol to form micelle and absorbed by the intestine. Saponins and pectin can also inhibit the absorption of fat and cholesterol that comes from food so fat and cholesterol can not be absorbed by the intestine and excreted through the feces (Dong *et al*., 2007).

Provision of alfalfa saponin extract effectively reduced abdominal fat deposition and increased the immunity of chicken but it can negatively affect the performance of broiler chickens. So, there should be a supplement that does not affect the performance of chickens (Dong *et al*., 2007).

Feed is one environmental factor that has high contribution on fat and cholesterol metabolism and precursors of cholesterol are obtained from feeds (Ismoyowati and Sumarmono, 2011). The present study indicates that feed intake of broiler chicken did not reduced by 5% powdered leaves of *P. buxifolius* because of the influence *P. buxifolius* which can inhibit leptin secretion. Leptin can reduce food intake and increase energy expenditure by binding and activating its specific receptor in the hypothalamus (Assal *et al*., 2007). High energy intake significantly stimulated leptin secretion in ewes (Towhidi *et al*., 2006). Leptin resistance might lead to reduced work efficiency of

leptin (Myers *et al*., 2008). Weight increase encouraged increased number of fat cells so that the mechanism of intake reduction is not happening even though levels of leptin in the blood increase (Newnham *et al*., 2002).

CONCLUSION

It can be concluded that the administration of powdered leaves of *P. buxifolius* as much as 5% can reduce the accumulation of intracellular fat, serum leptin levels and cholesterol broiler chicken meat. Leaves of *P. buxifolius* have great potential to reduce fat and cholesterol meat levels in broiler chicken.

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REFERENCES

AOAC, 1999. Official Method of Analysis of The Association of Analytical Chemists. 17th Edn., Association of Official Analytical Chemists, Washington DC.

Adeneye, A.A., O.O. Amole and A.K. Adeneye, 2006. Hypoglycemic and hypochlolesterolemic activities of the aqueous leaf and seed extract of *Phyllantus amarus* in mice. Fitoterapia, 77: 511-514.

Ahmed, R., S.J. Moushumi, H. Ahmed, M. Ali, H. Reza, W.M. Haq, R. Jahan and M. Rahmatullah, 2010. A study of serum total cholesterol and triglyceride lowering activities of *P. emblica* L. (Euphorbiaceae) fruits in rats. Advaces Nat. Applied Sci., 4: 168-170.

Ashwell, C.M., S.M. Czerwinski, D.M. Brocht and J.P. McMurtry, 1999. Hormonal regulation of leptin expression in broiler chickens. Am. J. Physiol., 276: 226-232.

Assal, H.S., M. Fath-Allah and A. Elsherbiny, 2007. Serum leptin and adiponectin in obese diabetic and non-diabetic. J. Medical Sci., 7: 865-869.

Bankole, H.A., O.A. Magbagbeola, O.B. Adu, A.A. Fatai and B.A. James, 2011. Biochemical Effect of Ethanolic Extract of *Phyllanthus amarus* (Euphorbiaceae) on Plasma Nitric Oxide and Penile Cyclic Guanosine Monophosphate (cGMP) in Mature Male Guinea Pigs Asian J. Biochem., 6: 291-299.

Boehringer, M.D., 1993. Enzymatic cholesterol high performance. CHOD-PAP KIT France SA. 38240.

Dong, X.F., W.W. Gao, J.M. Tong, H.Q. Jia, R.N. Sa and Q. Zhang, 2007. Effect of polysavone (alfalfa extract) on abdominal fat deposition and immunity in broiler chickens. Poult. Sci., 86: 1955-1959.

Goldstein, J.L., W.R. Hazzard and H.G. Schrott, 1973. Hyperlipidemia in coronary heart disease I. lipid levels in 500 survivors of myocardial infarction. J. Clin. Invest., 52: 1533-1543.

Gonzalez-Paramas, A.M., S. Esteban-Ruano, C. Santos-Buelga, S. De Pascual-Teresa and J.C. Rivas-Gonzalo, 2004. Flavanol content and antioxidant activity in winery byproducts. J. Agric. Food Chem., 52: 234-238.

Gopal, K. and S.K. Ranjhan, 1980. Laboratory manual of nutrition research. Mayapury Phase 1, A-49, New Delhi, India, pp: 135-138.

Guyton, A.C. and J.E. Hall, 2006. Texbook of Medical Physiology. 11th Edn., University of Mississipi Medical Center, Mississipi.

Harborne. J.B., 1996. Metode Fitokimia: Guide to modern ways to analyze the plant. D. Matter Interpreter, K. Padmawinata and I. Soediro. ITB-Bandung.

Hsu, C.L. and G.C. Yen, 2007. Effects of flavonoids and phenolic acids on the inhibition of adipogenesis in 3T3-L1 adipocytes. J. Agric. Food Chem., 55: 8404-8410.

Indra, R., T.E. Hernowati, Satuman and E. Widodo, 2010. Cultured adipocytes and inspection adipositokin. Physiology Laboratory Science, Brawijaya University, Fakulty of Medicine, Malang.

Ismoyowati and J. Sumarmono, 2011. Fat and cholesterol contents of local duck (*Anas platyrhynchos platyrhynchos*) meat fed mash, paste and crumble feeds. Asian J. Poult. Sci., 5: 150-154.

James, D.B., N. Elebo, A.M. Sanusi and L. Odoemene, 2010. Some biochemical effect of intraperitoneal administration of *P. amarus* aqueous extracts on normaglycemic albino rats. Asian J. Med. Sci., 2: 7-10.

Kim, J.H., S.Y. Lee, S.C. Myung, S.Y. Kim, T. Kim and M.K. Kim, 2008. Clinical significance of the leptin and leptin receptor expressions in prostate tissues. Asian J. Androl., 10: 923-928.

Kongo-Dia-Moukala, J.U., J.N. Atindana and H. Zhang, 2011. Hypocholesterolemic activity and characterization of protein hydrolysates from defatted corn protein. Asian J. Biochem., 6: 439-449.

Kuppusamy, U.R. and N.P. Das, 1994. Potential of β adrenoreceptor agonist-mediated lipolysis by quarcetin and fisetin in isolated rat adipocytes. Biochem. Pharmacol., 47: 521-529.

Lampino, F. and S.S. du Plessis, 2008. Insulin and leptin enhance human sperm motility, acrosome reaction and nitric oxide production. Asian J. Androl, 102: 799-807.

Lecumberri, E., L. Goya, R. Mateos, M. Alia, S. Ramos, M. Izquierdo-Pulido and L. Bravo, 2007. A diet rich in dietary fiber from cocoa improves lipid profile and reduces malondialdehyde in hypercholesterolemic rats. Nutrition, 23: 332-341.

Ma, Z., Y. Xu, Y. Zhang, L. Dong and Q. Li *et al*., 2010. Anti-metabolic syndrome effect of Licorice flavonoid dispersible tablets in a high fat diet-induced obesity/type 2 diabetes mouse model. Asian J. Tradit. Med., 55: 89-101.

Marounek, M., Z. Volek, A. Synytsya and J. Copikova, 2007. Effect of pectin and amidated pectin on cholesterol homeostasis and cecal metabolism in rats fed a high-cholesterol diet. Physiol. Res., 56: 433-442.

Maruthappan, V. and S. Shree, 2010. Effects of *Phyllanthus reticulatus* on lipid profile and oxidative stress in hypercholesterolemic albino rats. Indian J. Pharmacol., 42: 388-391.

Matsui, Y., H. Kumagai and H. Masuda, 2006. Antihypercholesterolemic activity of catechin-free saponin-rich extract from green tea leaves. Food Sci. Technol. Res., 12: 50-54.

Mohkam, M., S.H. Zarkesh-Esfahani and M. Fazeli, 2011. Construction of a recombinant fab fragment of a monoclonal antibody against leptin reseptor. Asian J. Biotechnol., 3: 493-506.

Morais, S.A.L., E.A. Nascimento, C.R.A.A. Queiroz, D. Pilo-Veloso and M.G. Drumond, 1999. Studies on polyphenols and lignin of *Astronium urundeuva* wood. J. Braz. Chem. Soc., 10: 447-452.

Murray, R.K., D.K. Granner, P.A. Mayes and V.W. Rodwell, 2003. Harper's Biochemistry. 20th Edn., Lange Medical Publications, California.

Myers, M.G., A.M. Cowley and H. Munzberg, 2008. Mechanisms of leptin action and leptin resistance. Ann. Rev. Physiol., 70: 537-556.

Nasri, H., S. Shirani and A. Baradaran, 2006. Lipids in association with leptin in maintenance hemodialysis patients. J. Med. Sci., 6: 173-179.

Newnham, J.P., T.J.M. Moss, I. Nitsos, D.M. Sloboda and J.R.G. Challis, 2002. Nutrition and the early origins of adult disease. Asia Pacific J. Clin. Nutr., 11: S537- S542.

Nurulhuda, M.H., A. Azlan, A. Ismail, Z. Amom and F.H. Shakirin, 2012. Cholesterol-lowering and artherosclerosis inhibitory effect of sibu olive in cholesterol fed-rabbit. Asian. J. Biochem., Vol. 7, In Press,

Perusse, L., Y.C. Chagnon, S.J. Weisnagel, T. Rankinen, E. Snyder, J. Sands and C. Bouchard, 2001. The human obesity gene map: The 2000 update. Obesity Res., 9: 135-169.

Qureshi, S.A., W. Asad and V. Sultana, 2009. The effect of *Phyllanthus emblica* Linn on type-II diabetes, triglycerides and liver-specific enzyme. Pak. J. Nutr., 8: 125-128.

Rodrigues, H.G., Y.S. Diniz, L.A. Faine, C.M. Galhardi and R.C. Burneiko *et al*., 2005. Antioxidant effect of saponin: Potential action of a soybean flavonoid on glucose tolerance and risk factors for atherosclerosis. Int. J. Food Sci. Nutr., 56: 79-85.

Roth, J.D., B.L. Roland, R.L. Cole, J.L. Trevaskis and C. Weyer *et al*., 2008. Leptin responsiveness restored by amylin agonism in diet-induced obesity: Evidence from nonclinical and clinical studies. Proc. Natl. Acad. Sci. USA, 105: 7257-7262.

Saez, F., A. Ouvrier and J.R. Drevet, 2011. Epididymis cholesterol homeostasis and sperm fertilizing ability. Asian J. of Androl., 13: 11-17.

Santoso, U., S. Ohtani and K. Tanaka, 2000. Tu-chung leaf meal supplementation reduced an increase in lipid deposition of chickens stimulated by dietary cholesterol. Asian Aust. J. Anim. Sci., 13: 1758-1763.

Shen, B., J. Yu, S. Wang, E.S. Chu and V. Wong *et al*., 2008. *Phyllanthus urinaria* ameliorates the severity of nutritional steatohepatitis both *in vitro* and *in vivo*. Hepatology, 47: 473-483.

Taleb, S., O. Herbin, H. Ait-Oufella, W. Verreth and P. Gourdy *et al*., 2007. Defective leptin/leptin receptor signaling improves regulatory T cell immune response and protects mice from atherosclerosis. Arteriosclerosis Thrombosis Vascular Biol., 27: 2691-2698.

Towhidi, A., H. Khazali, A. Niasari Naslaji and M. Zhandi, 2006. The effects of energy on the gonadotrophins secretion are mediated by leptin in ewes. Pak. J. Biol. Sci., 9: 2391-2401.

Ueda, H., 2001. Short-term feeding response in chicks to tea saponin. J. Poult. Sci., 38: 282-288.

Umbare, R.P., G.S. Mate, D.V. Jawalkar, S.M. Patil and S.S. Dongare, 2009. Quality evaluation of *Phyllanthus amarus* (Schumach) leaves extract for its hypolipidemic activity. J. Biolo. Med., 1: 28-33.

Wang, H.K., 1998. Plant-derived anticancer agents currently in clinical use or clinical trials. Investig. Drugs J., 1: 92-102.

Wang, M.Y., L. Chen, G.O. Clark, Y. Lee and R.D. Stevens *et al*., 2010. Leptin therapy in insulin-deficient type I diabetes. Proc. Natl. Acad. Sci., 107: 4813-4819.

Wardah, T. Sopandi and Wurlina, 2007. Identification of active compounds *P. buxifolius* leaf ethanol extract and its effect on preview serology and hematology of broiler chickens infected by the virus of Newcastle. J. Drug Nat. Mater., 6: 88-95.

Williams, K.W., M.M. Scott and J.K. Elmquist, 2009. From observation to experimentation: Leptin action in the mediobasal hypothalamus. Am. J. Clin. Nutr., 89: 985S-990S.

Xia, D., X. Wu, Q. Yang, J. Gong and Y. Zhang, 2010. Anti-obesity and hypolipidemic effects of a functional formula containing *Prumus mume* in mice fed high-fat diet. Afr. J. Biotechnol., 9: 2463-2467.

Zhang, L.Z., Y.J. Guo, T.U. Gz, W.B. Guo and F. Miao, 2000. Studies on chemical constituents of *Phyllanthus urinaria* L.. Zhongguo Zhong Yao Za Zhi, 25: 615-617.

Zunft, H.J.F., W. Luder, W. Harde, B. Haber, H.J. Graubaum, C. Koebnick and J. Grunwald, 2003. Carob pulp preparation rich in insoluble fibre lowers total and LDL cholesterol in hypercholesterolemic patients. Eur. J. Nutr., 42: 235-242.