Blood Profile of Layers in Response to *Sauropus androgynus* Leaves Extract Supplementation in Fermented Palm Oil Sludge Based Diet

Bieng Brata, Yosi Fenita, Nurmeiliasari, and Teguh Yogaswara

Animal Science Department, Faculty of Agriculture, University of Bengkulu
Jl. Raya Kandang Limun, Bengkulu Indonesia
Email: yosifenita@yahoo.co.id

ABSTRACT

The research was conducted to investigate the blood profile of layers in response to *Sauropus androgynus* leaves extract (SAE) supplementation in femented palm oil sludge (FPOS) based diet. A total of fifty layers were used in the experiment set up with completely randomized design of five different treatment groups. Dietary treatments evaluated were P0 (without additional FPOS and SAE), and the other four were inclusion of 15% FPOS as basal diet (P1), 15% FPOS + 3 gr SAE (P2), 15% FPOS + 6 gr SAE (P3), 15% FPOS + 9 gr SAE (P4) and 15% FPOS + 12 gr SAE (P5). The research was conducted for eight weeks and blood samples collection were taken at the end of the research to measure number of eritrocyte, haemoglobyn (Hb), packed cell volume (PCV), MCV, MCH and MCHC. Results showed that feeding a combination of FPOS and SAE suplementation had resulted in an insignificant different of Hb, PCV, and values of MCV, MCH and MCHC compared with the control group ($P>0.05$). In contrast, there was a significant different on the number of erytrocyte between control group and FPOS and SAE treatment groups ($P<0.05$). In conclusion, the SAE supplementation in FPOS based diet decreased the number of erytrocyte but did not affect the Hb, PCV, MCH, MCV and MCHC. A supplementation of SAE 12gr/Kg in FPOS based diet showed the best values of PCV and Hb.

Key words: Sauropus androgynus extract (SAE), fermented palm oil sludge (FPOS), blood profile, layers.

INTRODUCTION

Utilization of fermented palm oil sludge (FPOS) as animal feed is widely explored. Limitations in palm oil sludge utilization as poultry diet are high crude fibre content (11-32.69%), ash content (9-25%) and low concentration of amino acid content (Hutagalung, 1978); therefore, fermentation process is required (Baeker *et al*., 1981; Pasaribu *et al*., 1998; Sinurat *et al*., 1998; Purwadaria *et al*., 1999; Bintang *et al*., 2000). Fermentation technology changes the an-organic nitrogen into cell protein as well as produces hydrolytic enzymes to increase diet digestibility (Purwadaria, 1998). It is reported that fermentation process increase the utilization level of palm oil sludge from 5 % to 10% in broiler diet (Sinurat *et al*., 2000). Moreover, Sonaiya (1995) stated that fermented palm oil sludge is able to mix into diet up to 20-40%. A research by Fenita *et al.* (2010) revealed that feeding 15% of fermented palm oil sludge resulted in pale yolk colour.

Some research reported various results of FPOS crude fibre content due to its different analysis methods. Fenita *et al*. (2010) reported that nutritional values of FPOS which is fermented by using *Neorosspora crassa*are as the followings ; 23,45% of crude protein, 17,34% of crude fibre, 1774 Kkal/kgenergy (ME), Ca 1,32%, P 0,56%, and 9,45% fat.Another finding mentioned that nutritional value of FPOS fermented by using A*spergilus niger*are 22,07%crude protein, 18,6%energy, (ME) 1717kkal/kg, Ca 1,24% and P 0,65% (Sinurat, 2003).

Therefore, a*Sauropus Androgynus*Extract (SAE) supplementation is applied to provide enough essential amino acids and β-caroten in supporting productivity of layers. A combination of SAE supplementation in FPOS based diet may improve productivity as can be seen through blood parameters.

MATERIAL AND METHODS

Experimental Animal

The research was conducted for two months started from April 1st, 2014 to May 31st, 2014, which was located in Commercial Zone Animal Laboratory of Animal Science Department, Universitas Bengkulu. Fifty layers aged 7 months old were assigned to a completely randomized design experiment with five treatment groups. The experimental treatments were P0 (without additional FPOS and SAE), and the other four were inclusion of 15% FPOS as basal diet (P1), 15% FPOS + 6 gr SAE (P2), 15% FPOS + 9 gr SAE (P3) and 15% FPOS + 12 gr SAE (P4). The layers were kept in individual cages and allowed adjustment for two weeks prior the experiment.

Experimental Diets

Treatments of the research were the utilization of FPOS as basal diets and different levels of SAE supplementations.

The Process of Making Fermented Palm Oil Sludge

The process of making fermented palm oil sludge is started by adding aquadest (70%) into dry palm oil sludge. Then it is mixed well and warmed for 30 minutes to sterilize the materials. At room temperature, the materials are inoculated with 9% Neurospora sp by mixing them well and incubated for 7 days (aerobic process for 5 days and an aerobic process for two days). After that the product of the fermented palm oil sludge is harvested, dried and grinded (see figure 1). The ration is tested for water content, crude protein, crude fibre, energy and fat. A fifteen per cent of FPOS is mixed into diet (Fenita *et al*, 2010).

The Process of Making SAE

Sauropus androgynus is extracted through serial of processes. Firstly, the leaves are wind dried and are boiled in 60^{O}C fresh water for 30 minutes with water *Sauropus androgynus* leaf ratio of 1:5. Secondly, it is then filterred and the filtrate is collected for next process use. Finally, it is boiled at the temperature of 60^{o}C for 12 hours; the extract is completed as it is thickening.

Blood Parameters

The blood collections taken from vena axiliaris were executed at day 60th of the experiment. The sampels were put into a heparinized-vacutainer before being analyzed for erytrocyte number, Hb and PCV. The measurements of erytrocyte was counted by using Improved Neubauer, PCV was performed by using haemocytometer and Hb was measured by using Haemoglobinometer Sahli.

Statistical Analysis

The collected data was analyzed statisticaly by using ANOVA procedure of costat. After a significant F test ($P<0.05$), the data was tested by using Duncan's multiple range test (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

Number of Red Blood Cell (RBC)

The treatments of inclusion FPOS and SAE had significantly lower RBC compared to control group ($P<0.01$) as shown in Table 4. Within the four groups treated with FPOS and SAE, the highest number of RBC was found in P4 (diet+15% FPOS+12 grSAE/Kg) (1.92±0.04 x 106); in contrast, the lowest was in P3 (diet+15% FPOS+12 grSAE/Kg) (1.72±0.15x 106) as shown in Table 1. These finding showed that a normal RBC number was only in control group (2.3±0.11) while the other four groups were below normal range of RBC. A normal RBC number in chicken is 2,0-3,2 x 106/mm3 (Mangkoewijodjo dan Smith, 1988). A decrease in RBC number, the main constituent of PCV, during egg production may due to antagonist pleiotropic effect of estrogen as reported by Wagner et al. (2004). Similar findings are reported by Khan and Zafar (2005) as a higher dose of estrogen administration had caused a significant decrease on RBC count. A study by Apata (2004) revealed that antinutrition factor in Prosopis Africana Seed had decreased number of RBC and depressed egg production of layers. Health might be another factor caused a lower RBC number. A diarrhoea case was observed in P2 and P3 groups during the 2nd week of the experiment.

Haemoglobin Values

The effects of different levels of SAE supplementation on PFOS based diet on haemoglobin values are summarized in Table 5. Hb values of layers control group was insignificantly different compared to P4 group of treatments (P<0.05). In addition, feeding FPOs and SAE in P1, P2and P3 groups had significantly lower Hb values of 7.1, 6.97, 6.7 gr/dl, respectively compared to control group(8.97 gr/dl) (P<0.05). It is important to note that there was insignificant difference among treatment group of P1, P2 and P3 (P>0.05). There was an increase of RBC concentration due to SAE supplementation which was followed by an increase in Hb concentration. This pattern showed that a higher level of SAE supplementation affected in a higher concentration of RBC as well as Hb. These results showed that feeding FPOS of 15%+12g/kg SAE had resulted in an increase in Hb values compared to group given only FPOS as basal diet. A normal range of Hb values is 7.3-10.9 gr/dl (Mangkoewidjodjo dan Smith, 1988).

Furthermore, Hb measurements in P0, P1 and P4 presented normal values; however, P2 and P3 groups which had SAE supplementation of 6 and 9 gr/kg tended to decrease Hb values in layers. These results are in agreement with a research by Wagner *et al.* (2008). Laying period might have caused a decrease in Hb values as this stage needs a huge amount of energy and or nutrients with some negative consequences on animal physiology. This study suggested that the decrease in hematocritobserved during egg production in birds might be due to antagonistic pleiotropic effects of estrogens.Unlike androgen, estrogen is known for its negative effects on erythropoesis (Sturkie et al., 1965). A decrease in Hb may occur if there is problem in eritropoiesis process of which the Iron (Fe) needed (Haper et l., 1985). Moreover, an interference in amino acids synthesis may also cause a problem in Hb formation (Guyton, 1982; Schlam *et al*., 1986). Health factor might have been an affecting factor in low values of Hb.Some of the experimental layers experienced diarrhoea during the research period.

Hematocrit (PCV)

Hematocrit (PCV) represents as a percentage of RBC in whole blood after a centrifugation (Swenson 1984). PCV values represent amount of oxygen in circulatory system (Cunningham, 2002). Furthermore, this study found that hematocriteaffects blood viscocity.

Results of ANOVA showed that P0 (29.67%) was not different from P4 (25.67%) in terms of PCV levels (P>0.05). Supplementation of 12 g/Kg SAE in FPOS based diet was able to subtitute commercial diet as it gave better PCV than that of FPOS diet. Similar with ANOVA results of previous parameters, PCV levels of control groups were significantly different from P1(24.00%), P2 (29.67%), and P3 (23.00%). A normal PCV range of mature hen is varied from 29% (Strukie,1965) to 33% (Sweanson,1970).

MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobine) and MCHC (Mean Corpuscular Haemoglobine Concentration)

SAE supplemetation in FPOS based diet did not significantly affect MCV values (P>0.05). The highest value of MCV was at control group (128.33±2.52) ; in contrast, the lowest was at 15% FPOS and SAE 9 gr/kg treatment group. The range of MCV values measured in the research was in a normal range. Some researches revealed that normal value of MCV is from 90 to 140 (Schalm *et al*., 1986).

Data of MCH is presented in Table. 5. Supplementation of different levels of SAE in FPOS based diet did not significantly affect MCH of all treatments. The range of MCH values is from 39.33 to 40. Jain (1993) reported similar values of MCH which range from 33.0 to 47.0.

Insignificant results of analysis of variance of MCHC values showed that a combination of SAE and FPOS did not give a negative impact to blood profile. These results are in agreement to a finding reported by Shalm (1986), that stated a normal range of 26.0-35.0.

CONCLUSION

SAE supplementation in FPOS based diet decreased the number of erytrocyte but did not affect the Hb, PCV, MCH, MCV and MCHC. A supplementation of SAE 12gr/kg in FPOS based diet showed the best values of PCV and Hb.

REFERENCES

Apata, D.F. 2004. Egg Production and hematology profile of laying hens fed dietary raw and processed Prosopis Africana seeds. Journal of Agriculture Research and Development. Pp.99-104

Bintang, I.A.K, A.P. Sinurat, dan T.Purwadaria. 2003. Respon broiler terhadap pemberian ransum yang mengandung lumpur sawit fermentasi pada berbagai lama penyimpanan. JTIV8(2):71-75

Cunningham, J.G. 2002. Textbook of Veterinary Physiology. USA. Saunders Company.

Dallman, H.D. 1990. *Buku Teks Histologi Veteriner II*. Third Edition. Alih bahasa : R. Hartono. Penerbit UI. Jakarta.

Darise, M. and Sulaeman. 1997. Ekstraksi komponen kimia daun katuk asal sulawesi selatan berbagai metode serta penelitian daya hambat terhadap bakteri uji. Warta Tumbuhan Obat, 3 (3): 37-38.

Fenita, Y., U. Santoso, H. Prakoso dan Iryun. 2010. Pemanfaatan Lumpur Sawit Fermentasi dengan *Neurospora sp* terhadap Performans Produksi dan Kualitas Telur. Jurnal Ilmu Ternak dan Veteriner. Pusat Penelitian dan Pengembangan Peternakan. Bogor

Ganong, W.F. 1993. *Review of Medical Physiology* 6th Ed. Prentice-Hall International Inc. San Fransisco.

Ganong, W.F. 2003. *Review of Medical Physiology*. International Edition. Mc Graw Hill Book. San Francisco.

Handerson JA, Blood DC. 1975. *VeterinerMedicine*. 4th Ed. Balltimore.The Williamsand Wilkins Company.

Hulshoff, P. J. M., C. Xu, P, Van De Bovencamp, Muhilal and C. E. West 1997. Application of a validated method for the determination of provitamin A carotenoids in Indonesian foods of different maturity and origin. *Jurnal Agriculture Food Chem.* 45: 1174-1179.

Khan, T.A and Farhat Z. 2005. Haematological study in response of varying doses of estrogen in broiler chicken. International Journal of Poultry Science 4 (10)748-751.

Malik, A. 1997. Tinjauan fitokimia, indikasi penggunaan dan bioktivitas daun katuk dan buah trengguli. Warta Tumbuhan Obat Indonesia. III (7) 39-41.

Nuraini, Sabrina and S.A. Latif 2008. Performa ayam dan kualitas telur yang menggunakan ransum mengandung onggok fermentasi dengan neurospora crassa. Media Peternakan. Universitas Andalas: 195-201.

Paik, I. K. 2001. Application of chelated mineral in animal production. Asian-Aust. J. Anim. Sci. 14(special issue) : 191-198.

Piliang, W.G., A. Suprayogi, N. Kusmorini, M. Hasanah, S. Yuliani, dan Risfaheri . 2001. Efek pemberian daun katuk (S*auropus androgynus*) dalam ransum terhadap kandungan kolesterol karkas dan telur ayam lokal. Lembaga Penelitian Institut Pertanian Bogor Bekerjasama dengan Badan Penelitian dan Pengembangan Pertanian. Proyek ARMP II. Desember 2001.

Santoso, U., Setianto, J dan Prakoso, H. 1999. Peningkatan efisiensi pertumbuhan dan penurunan jumlah *Salmonela sp.* daging serta akumulasi lemak broiler oleh ekstrak daun katuk. Laporan Penelitian. Departemen Pendidikan dan Kebudayaan, Universitas Bengkulu, Bengkulu.

Santoso, U dan Sartini. 2001. Reduction of fat accumualation in broiler chickens by *Sauropus androgynus* (katuk) leaf meal supplementation. Asian-Aust. J. Anim. Sci. 14:346-350.

Santoso, U., Setianto, J., Suteky, T. dan Fenita, Y. 2003. Penggunaan ekstrak daun katuk untuk meningkatkan efisiensi produksi dan kualitas telur yang ramah lingkungan pada ayam Leghorn. Laporan Penelitian Hibah Bersaing Lanjutan. Lembaga Penelitian Universitas Bengkulu. Bengkulu.

Santoso, U., Setianto, J. dan Suteki, T. 2005. Effect of *Sauropus androgynus* extract on egg production and lipid metabolism in layers. Asian-Aust. Jurnal Animal Science. Vol 18 (3): 364.

Schalms OW, Jain NC, Carol EJ. (1986). *Veterinary Haematology*.4th Ed. Philadelphia. Lea and Febiger.

Sinurat, A.P. 2003. Pemanfaatan lumpur sawit untuk bahan pakan unggas. Wartozoa. Buletin Ilmu Peternakan Indonesia. Vol 13(2)39-47.

Soeharsono.1976. Respons broiler terhadap berbagai kondisi lingkungan. Disertation of Post Graduate School of Universitas Padjadjaran. Bandung.

Struki PD.1976. Avian physiology. 3 rd Ed. New York. Comstock Publishing Associates A Devision of Cornell University Press Ithaca.

Wagner, E.C., J.S. Prevolsek, K.E. Wynne-Edwards and T.D. Williams. 2008. Hematological changes associated with egg production: estrogen dependence andrepeatability. The Journal of Experimental Biology 211, pp. 400-408.

Yulianis, S. dan T. Marwati. 1997. Tinjauan katuk sebagai bahan makanan tambahan yang bergizi. Warta Tumbuhan Obat, 3 (3): 55-56.