The Effects of Amofer Palm Oil Waste-based Complete Feed to Blood Profiles and Liver Function on Local Sheep

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Abstract—**Amoniation-Fermentation (amofer) technology should be conducted in order to improve the low quality of by product produced from palm oil plantations and mills (palm oil waste) which is used for constituent of feed ingredients in complete feed (CF). This technology also reforms the feed material into edible form. Before broadly applicable, it must be ensured that the feed does not have toxic effects on livestock. This research was peformed to evaluate the effects of amofer palm oil waste-based CF to blood profile and liver function on local sheep. Completely Randomly Design (CRD) was used with 4 treaments and 4 replications. The observed variables were the levels of hemoglobin, hematocrit, blood glucose, ALT and AST was analyzed by ANOVA. The average value of blood glucose levels at T_1= 80.68 mg/dl, T_2=79.08 mg/dl, T_3=81.18 mg/dl and T_4=73.70 mg/dl. The average value of hemoglobin levels at T_1=10.80 g/dl, T_2=10.30 g/dl, T_3=11.23 g/dl and T_4=10.25 g/dl. The average value of hematocrit levels at T_1=31.00%, T_2=31.00%, T_3=33.75% and T_4=30%. The average value of ALT levels at T_1=17.90 µl, T_2=13.83 µl, T_3=18.75 µl and, T_4=13.40 µl. The average value of AST level at T_1=106.20 µl, T_2=88.98 µl, T_3=104.40 µl and T_4=91.25 µl. There was no significant difference among four treatments (p>0.05). The administration CF did not cause hematological disorders which showed by the blood profiles and liver function were in normal range, so that suggested the CF was appropriate and safe for local sheep.**

[*Keywords—amofer, complete feed, hemoglobin, hematocrit, glucose, liver function*]

I. INTRODUCTION

The competitiveness of feed industry is defined from the availability of raw material as well as breeding**,** management, livestock health and technology. The improvement of livestock's business unit grows in feed center may necessary take the advantage from its byproduct or waste as a useful resources but haven't optimized yet. The low quality of agriculture, plantation and agroindustry byproducts still left an issue which is needs to be solved. One of the technologies to deal with is amofer which applies before the feed is formulated into complete feed. Complete feed (CF) is equal rations fed for livestock to support the life's needs, growth and production without any feed addition except water. In nutrition life's cycle, CF has more benefit efficiency than separated feed (Phillips, 2001; Hardianto, 2003; Utomo, 2004; Mayulu, 2009).

The development of CF technology is a method or technique of ruminant's feed production where forages and concentrate or byproduct of agriculture, plantation or agroindustry which haven't optimized yet is homogenous mixed through physically, chemically or biologically processing and also employs hydrolysis, fermentation and ammonization (Verma *et al*., 1996; Mathius, 2008). The CF technology is a "feeding strategy" alternative for beef livestock which can be broadly a pplied in numerous conditions of
livestock area. The provision of feed as CF is considered has more effective and efficient which compare to farmer who used to provide separated forages and concentrate. CF can be fed together with forages and packaged concentrate which has more complete nutrition value, quality and more practice even in time based factor for livestock, worker (Sunarso, 2003; Wahyuni and Bijanti, 2006; Kusnadi, 2008; Haryanto, 2009).

Palm waste is local biomass which has high potency as CF constituent but the utilization is under optimized. The positive side is that palm waste doesn't compete with others animal, human, easy to obtain, abundant of supply, available along a year, appropriate palatability, safe for livestock health and cheap. Palm waste which can be in the form of steam, leaf, empty bunch, fruit pressed fiber, empty fruit bunch and sludge is a potential feed alternative at 60–70% for goat. The CF function of palm oil by products is obtained through processing and additive substance to achieve the optimal nutrition value and can be consumed by animal. The palm frond and leave is technically inefficient feed material due to the low protein value. This material needs to be physically processed and should not be given to cattle in single feed as it is less preferred (Sutrisno, 2002; Batubara, 2003; Mathius, 2008).

Several previous researches showed the significant benefit of amofer technology for CF formula, such as feed efficiency, feed conversion, body weight increment and animal productivity (Sunarso, 2003; Mayulu, 2009). The toxic effect of amofer-based CF towards animal systemic function was rarely found. The objective of this research was to evaluate the effect of palm oil waste-based CF using amofer technology to blood profile and liver function of local sheep.

II. RESEARCH METHOD

The research was conducted at Laboratory of Animal Nutrition and Feed Science, Diponegoro University,

Semarang, Indonesia. The objects study was 16 male local sheeps, age at 9 months, weight at 14.82±0.82 kg (CV=5.52%) which then divided into four groups. All sheep was put into individual cages. The treatments was palm oil waste-based CF using amofer technology at crude protein (CP) 10.63% (T_1), 12.27% (T_2), 13.70% (T_3) and 15.90% (T_4) with *Total Digestible Nutriens* (TDN) at 61.83–64.21%.

The first feed processing was ammonization and fermentation of feed material produced from palm tree (i.e. frond, leave, empty bunch and fruit pressed fiber). Ammonization used urea 3% of material weight and ripened up to 18 days. Fermentation used organic substance microbe produced by Kurnia Makmur Veteriner at 1% and ripened up to 18 days. The second was milling process to reduce particle size of material and followed by mixing process of all material constituent (Table 1) using mixers to obtain CF at concentrate form and mesh size. The third was proximate analysis to obtain nutrition value before it was applied into treated sheep.

The adaptation period of CF treatment was 7 days. Blood sampling was conducted after sheep consuming CF for 37 research days which was taken using vena jugularis at 10 ml. The blood sample for glucose analysis was put into tube filled with NaF anticoagulant; put into tube filled with EDTA for hemoglobin and hematocrit analysis; while for ALT and AST analysis, anticoagulant was added and let it idle approximately at 30 minutes and then centrifuged at 3000 rpm for 15 minutes and then took the serum. All samples were put into ice thermos and clinic pathology laboratory investigation was immediately conducted (Salasia and Khusnan, 2001; Anele *et al*., 2010; Braun *et al*., 2010).

The research used complete randomized design with four replications. The observed parameters were blood profile i.e. hemoglobin, hematocrit, glucose and liver function i.e. *alanine aminotransferase* (ALT) or *Serum Glutamic Pyruvic Transaminase* (SGPT) and *aspartate aminotransferase* (AST) or *Serum Glutamic Oxaloacetate Transaminase* (SGOT). Analysis used ANOVA with significance level was prefereed if $p<0.05$ with 95% confidence interval.

III. RESULT AND DISCUSSION

In order to increase the quality of waste obtained from plantation and palm oil for CF material, processing technology using amofer is needed. This technology is important to change the form of waste into appropriate edible feed. To obtain maximum utilization, CF used in this research was constituted from ammoniated and fermented palm oil waste and added with others feed material in equal composition. The composition and formula of CF nutrient is shown in Table 1. Ammonization is an alkali treatment applied in fiber feed to increase the digestibility and nitrogen (N) component using urea. The main objective of this technique is to cut the lignin bond and release cellulose and hemicellulose (Sutardi, 1997; Sunarso, 2003; Van Soest, 2006).

The processing of palm oil waste used for feed material agreed with previous research. The treatment of silage and ammonized palm oil leave for sheep could increase dry matter content, organic substance, NH_3, PH and improved the value of ration consumption, digestibility (dry matter, organic substance and NDF) and also gave positive value of nitrogen balance and energy. Solid palm oil waste in CF block form is potential livestock nutrient resource because it consists of rough protein value at 12.63% and energy 154 kal.100 gram significantly increased the body weight increment and safe for livestock (Hanafi, 2004; Utomo, 2004).

TABLE 1.
THE COMPOSITION AND NUTRITION OF COMPLETE FEED FORMULA USED IN THE RESEARCH

Composition	Treatments			
	T_1	**T_2**	**T_3**	**T_4**
Feed material:	**(%)**			
Palm stem (amofer)	5.00	9.50	11.60	5.00
Palm leaf (amofer)	1.00	2.10	3.00	13.40
Empty bunch (amofer)	4.00	5.00	4.00	3.00
Fruit pressed fiber (amofer)	17.00	6.00	4.00	3.00
Palm oil sludge	1.00	4.00	6.00	5.00
Palm oil bunch	10.50	10.00	10.00	4.50
Legum	0.50	2.00	0.50	5.50
Corn	14.00	7.00	9.00	16.00
Rice bran	5.00	19.10	29.00	26.00
Cassava waste pulp	40.00	33.00	20.40	16.00
Molasses	0.50	0.50	0.50	0.50
Urea	-	0.30	0.50	0.60
Mineral mix	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Feed nutrient:				
Dry matter[1]	84.34	84.49	83.21	82.00
Organic material[1]	90.09	88.31	86.69	87.31
Crude protein[1]	10.63	12.27	13.70	15.90
Rough fat[1]	2.00	2.09	2.40	2.18
Rough fiber[1]	22.58	24.90	22.53	25.19
BETN[2]	54.88	49.05	48.05	44.04
TDN[2]	63.65	61.83	64.21	62.13
Ca[3]	0.43	0.34	0.31	0.30
P[3]	0.20	0.24	0.27	0.21

[1] Proximate analysis obtained from Laboratory of Animal Feed Science, Faculty of Animal Science, Diponegoro University
[2] Calculation result based on Sutardi (2001)
[3] Analysis result obtained from Laboratory of Biochemical, Faculty of Animal Science, Diponegoro University

The development of CF is expected to support balanced feed provision as it has balanced nutrition due to the function of fiber resource and concentrate. Therefore, aside from its composition consideration, CF should have appropriate nutrition value in order to fulfill the life needs of livestock.

Based on nutrition perspective, feed material is an important component to support the main life's needs, growth, production and or animal reproduction. Good feeding enable livestock to maintain normal metabolic function, balanced body tissue and produce energy to perform the metabolic process (Judge *et al*., 1989; Salfina *et al*., 2004). The balanced body tissue and systemic function livestock performance test of CF processed by amofer technology can be measured using blood profile i.e. glucose, hemoglobin and hematocrit content and also liver function i.e. ALT enzyme and AST content. Blood profile measurement is so important because of the vital role of blood function for all life substance and also to monitor and evaluate disease or animal disorders.

Glucose is formed from glycogenic compound which experienced gluconeogenesis. This compound is divided into two categories: 1) compound which conducts direct net conversion into glucose without involving major recycle process such as amino acid and propionate and 2) compound as a product of glucose partial metabolism at particular tissue

which transported to liver and kidney to be synthesized into glucose. In ruminant, glycogenic propionate acid is absorbed from rumen to portal circulation and then transported to liver to be changed into glucose form. The provision of high concentrate in feeding increases the feed material ferment ability in rumen. Concentrate has higher carbohydrate which easy to be fermented in rumen so the proportion of propionate acid and glucose content in blood becomes higher (Tillman *et al*., 1998; Murray *et a*l., 2003).

The average value of glucose content in treated sheep blood with amofer palm oil waste-based CF under different protein content was at range of 73.70–81.18 mg/dl (Table 3). For a comparison, the glucose content in healthy sheep was 44–81.2 mg/dl (Fraser *et al*., 1988). It showed that the glucose content obtained in this research still in normal range and did not significantly different among treatments (p>0.05). The provision of concentrate added with 4.5% urea of its concentrate content did not show toxic symptoms. The blood glucose of sheep was lower than mammal and birds. This agreed that ruminant will ferment carbohydrate into volatile fatty acid (VFA) which then replaces glucose as the main resource of tissue metabolism (Utomo, 1996; Fraser *et al*. 1988; Murray *et al*., 2003).

In ruminant which fed by conventional feeding, glucose will not be fermented in rumen and will be directly absorbed in unbroken form. The total glucose which is absorbed by body is divided into 15% synthesized in kidney and 85% in liver. The addition of urea in feeding is not able to change metabolism of rumen microorganism in a short time due to an energy competition between urea synthesis and gluconeogenesis. It was expected that urea addition and ammonia formation in rumen enable to increase urea synthesis in liver in order to decrease the gluconeogenesis number (Emmanuel, 1981; Emmanuel and Edjtehadi, 1981).

Glucose experiences catabolism through two formations i.e. glycolytic in anaerobic condition which produces pyruvate acid and Krebs cycle in aerobic condition which produces energy in the form of *adenosin triphosphat* (ATP) in mitochondria (Emmanuel *et al*., 1982; Arora, 1995; Murray *et al*., 2003). The failure of glucose utilization in animal fed with urea inside the feeding might be caused by these factors: 1) the blockage of blood ammonia during synthesis or insulin secretion; 2) the damage of cell membrane which is caused by bad effect of utilization and glucose transportation; and 3) the blockage of Krebs cycle and oxidative phosphorylation (Emmanuel, 1980; Emmanuel, 1981).

Hemoglobin (Hb) is the main component of red blood cell, such kind of protein substance which has molecular weight 64.450. Hemoglobin is a round molecule which is formed by four sub units. Each sub units consists of a polypeptide. Heme is porphyrins derivate which consists of iron. Hemoglobin synthesis in red blood cell is carried out from erythroblast up to stadium of reticulocyte growth. The main function of hemoglobin is to bind oxygen and release it into cells and tissue around the body for metabolic process. The capability to bind oxygen exists at high partial pressure. While in low partial pressure, the oxygen is released and transferred into cells (Murray *et al*., 2003).

Blood hemoglobin concentration is measured based on color intensity using photometer and expressed in gram hemoglobin/hundred milliliter blood (g/100 ml) or gram/deciliter (g/dl). Hemoglobin content is influenced by feed sufficiency particularly protein in ration, digestibility, age, sex and cattle species (Schalm *et al*., 1986). The normal range of hemoglobin content in male sheep is 11 g/dl (Tambuwal *et al*., 2002). In this research, the average value of hemoglobin was 10.3-11.25 g/dl (Table 2). Difference test showed that p>0.05 which defined that CF treatments under different protein content did not cause significant difference toward treated sheep. The increment of hemoglobin value was in normal range which indicated that amofer process did not create a problem occurred in blood circulation.

TABLE 2.
THE AVERAGE VALUE OF SHEEP BLOOD PROFILE TREATED WITH *COMPLETE FEED*

Treatment	Blood Glucose (mg/dl)	Hemoglobin (g/dl)	Hematocrit (%)
T_1	80.68 ± 4.64	10.80 ± 0.98	31.00 ± 1.63
T_2	79.08 ±7.36	10.30 ± 1.39	31.00 ± 2.16
T_3	81.18 ± 6.90	11.23 ± 0.58	33.75 ± 0.96
T_4	73.70 ± 0.62	10.25 ± 1.17	30.00 ± 2.16

Hematocrit or packed volume cell (PCV) is determined as a whole blood volume which consists of red blood cell obtained after blood specimen is centrifuged and is expressed in millimeter cubic packed cell/100 ml blood or in volume/100 ml (Price and Wilson, 1995). According to Taiwo and Ogunsanmi (2003), the normal hematocrit value for healthy sheep was 36–37%, while Orheruata and Akhuomobhogbe (2006) stated that the range was 18–38%. The increased of body dehydration could increase hematocrit value, while feeding with less nutrition causes less blood formation and decrease hematocrit value (Frandson, 1992).

The average value of hematocrit obtained in this research was 30–33.75% (Table 2). Difference test among treatments showed that (p>0.05). This value showed that the increment of protein content did not cause significant difference toward hematocrit and CF processed by amofer technique which in other words it suggested that treatments did not cause bad effect in circulation system (blood profile) in treated sheep. This result also indicated that applied CF had sufficient nutrition so that treated sheep did not experience anemia or dehydration. Anemia is a condition lack of erythrocyte, hemoglobin quantity and erythrocyte volume (hematocrit) per 100 ml blood. So, anemia is not a diagnosis but it reflects a pathophysiology change occurred in body (Price and Wilson, 1995).

Liver is parenchyma organ which is considered as the main organ having complicated and numerous functions. Liver is so important to maintain life and role metabolism function of carbohydrate, protein and fat. Some of glucose is metabolized in tissue to produce heat and energy. Liver also enables to synthesized glucose from protein and fat (gluconeogenesis). Amino acid degradation is started in liver organ through deamination process. Released ammonia is then synthesized into urea and excreted by kidney and intestine. In liver, ammonia produced in intestine by bacteria consisted in protein is transformed into urea. Others liver functions are to conduct cytogenesis, cholesterol and phospholipid synthesis, gall synthesis and secretion, bilirubin

assimilation and detoxification (Price and Wilson, 1995; Danfaer, 1994).

The increment of liver parenchyma cells disorder because of injury, necrosis or membrane permeability can increase enzyme content produced by liver such as ALT and AST which includes as transaminase enzyme (aminotransferase). ALT is also called as *glutamate pyruvate transaminase* (GPT), which mainly produced in liver. ALT enzyme catalyzed amino groups transfer between L-alanine and glutamate for physiologist needs. AST enzyme is called as *glutamate oxaloacetate transaminase* (GOT) which catalyzes amino groups and ketone between α amino acid and α ketone acid. This enzyme is usually produced in heart, body muscle, brain and lungs, less of it is found in liver (Pratt and Kaplan, 2000; Rochling, 2001; Murray *et al*., 2003).

ALT enzyme is cytosol enzyme/cytoplasm; if there is a permeability disorder of liver cell membrane will cause cytoplasm component enter the blood circulation which causes increment enzyme content in serum. AST enzyme is bound to mitochondria, so heavy cell damage will cause this enzyme enters blood circulation and increase the content (Rochling, 2001; Murray *et al*., 2003). The normal range of this enzyme at sheep is ALT 14.8–43.8 µl and AST 49.0–123.3 µl (Fraser *et al*., 1986). The average value of ALT and AST on treated sheep presents in Table 3. The value of ALT and AST was 13.40–18.75 µl and 91.25–106.20 µl, respectively which determined that liver function was in normal range. Application of CF with protein level difference did not cause significant difference ($p>0.05$) and amofer technique as well as urea additive did not cause liver function disorder at treated sheep.

TABLE 3.
THE AVERAGE VALUE OF ALT AND AST CONTENT OF TREATED SHEEP

Treatment	ALT (µl)	AST (µl)
T_1	17.90 ± 2.08	106.20 ± 34.45
T_2	13.83 ± 2.84	88.98 ± 5.56
T_3	18.75 ± 2.35	104.40 ± 26.71
T_4	13.40 ± 8.23	91.25 ± 22.57

The increased of ALT enzyme is directly related with liver cells damage if injured or inflammation is occurred. While AST enzyme in serum will increase if damage occurs in mitochondria and the content is directly related with the damage area of tissue. The content of ALT increases fast if liver is damaged with hepatitis, sirosis, tumor and icterus or hepatotoxic because of medicine consumption. The value of AST increases at almost liver disorder function, including non-hepatic function such as infarct myocardia, aritmia and kidney necrosis. This value will extremely increases (>1000) at heavy liver necrosis, heavy infarct disorder and muscle bone disorder (Price and Wilson, 1995; Rochling, 2001).

IV. CONLUSION

The administration CF did not cause hematological disorders which showed by the blood profiles and liver function were in normal range, so that suggested the CF was appropriate and safe for local sheep.

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