

Doi: 10.21059/buletinpeternak.v43i4.44606

Diet with Concentrate Containing *Durio zibethinus* Murr Seed Meal: Nutrient Contents, Fatty Acid Profiles, *In Vitro* Characteristics, and Nutrient Digestibility in Dairy Cows

Endang Sulistyowati^{1*}, Irma Badarina¹, Sigit Mujiharjo², Tamrin Simbolon¹, and Idop Rohani Purba¹

¹Department of Animal Science, Faculty of Agriculture, University of Bengkulu, Bengkulu, 38371, Indonesia

²Department of Agriculture Technology, Faculty of Agriculture, University of Bengkulu, Bengkulu, 38371, Indonesia

ABSTRACT

This research was to evaluate concentrate diet containing *Durio zibethinus* seed meal (DSM) on nutrient contents, fatty acid profiles, *in vitro* characteristics, and nutrients digestibility in dairy cows. The experiment was arranged in Latin Square 4 x 4 design with 4 lactating dairy cows in 4 periods in 2 weeks for each period. The treatments were diets with concentrate containing ratios of rice bran and DSM, DC 35/12.5 (35% of Rice bran+ 12.5% of DSM), DC 27.5/20 (27.5% of Rice bran+ 20% of DSM), DC 20/27.5 (20% of Rice bran+ 27.5% of DSM) and DC 12.5/35 (12.5% of Rice bran+ 35% of DSM). Data were analyzed using analysis of variance (Anova), if any significant difference among treatment means were found, will be further analyzed using Duncan Multiple Range Test (DMRT). Results showed that 20% of DSM supplemented in concentrate improved nutrient contents of crude protein, ether extract, medium chain fatty acid (MCFA), long chain fatty acid (LCFA), polyunsaturated fatty acid (PUFA), unsaturated fatty acid (UFA), and n6/n3. *In vitro* dry matter digestibility, organic matter digestibility, N-NH₃, and total VFA were high in concentrate containing 35% DSM. However, *in vivo* digestibility evaluation showed nutrient intakes were no differences among treatments. Nutrient digestibility were found higher in diet with concentrate containing 27.5% of DSM. There were three different optimal levels could be suggested, 20% of DSM (for nutrient and fatty acid content), 27.5% of DSM (for nutrient digestibility), and 35% of DSM (for *in vitro* characteristics). Supported by previous milk production, it was concluded that the 27.5% of DSM was suggested to be applied in concentrate for dairy cows.

Article history

Submitted: 29 March 2019

Accepted: 4 October 2019

* Corresponding author:

Telp. +62 81227675179

E-mail: ensulistyowati@yahoo.com

Keywords: Concentrate diets, Dairy cows, Digestibility, *Durio zibethinus* seed meal, Fatty acids

Introduction

Concentrate plays an important role in feeding ruminant animals. Feed ingredient, such as by-product of Durian (*Durio zibethinus* Murr), seed, that is abundant in Bengkulu and some other places, such as in most provinces in Sumatera, in Indonesia, can be utilized as one of the feedstuffs composed in concentrate. The production of this fruit in Bengkulu is about 15.319 ton/yr or 1.8% of its production in Indonesia, 847,489.6 ton/yr (Kementerian Pertanian, 2017).

Durio consists of 20-25% fruit meat, 5-15% seed, and 60-70% peel (Suhaidi, 2014). If it all can be collected together, there will be a potency of 85.92 ton/yr seed that can be converted as concentrate ingredient for ruminants, such as dairy cow and dairy goat. *Durio zibethinus* seed meal contained 6.05% protein, 0.41% ether

extract, and 1.82% crude fiber (Sistanto *et al.*, 2017). This seed meal can be incorporated into concentrate. Nuraini *et al.* (2015) reported on the utilization of Durio waste (seed and peel) fermented by using a mixture of *Phanerochaete chrysosporium* and *Neurospora crassa* with the same ratio in 8% inoculum dose for 9 day incubation was considered the optimal one in improving nutrient content. Beside the usage of Durio waste, other ingredients composed in concentrate were the source of fat, yeast, curcuma, and rice bran to improve nutrient content and rumen metabolism.

Polyunsaturated fatty acid (PUFA) source (2.11% of corn oil) in concentrate was considered the optimal level for milk production and health of dairy goats (Sulistyowati *et al.*, 2013); 0.5% yeast and 2% *Curcuma xanthorrhiza* Roxb powder in concentrate for dairy goat showed stable nutrients

during six weeks of storage (Sulistyowati *et al.*, 2015) and had high volatile fatty acid (VFA), yet low N-NH₃ in *in vitro* of goat rumen (Sulistyowati *et al.*, 2014).

Yeast supplementation has been known to affect rumen metabolism in certain ways. Some research results showed that yeast supplementation in standard, acidogenic and grazing did not show any significant effects in dairy cows (Ambriz-Vilchis *et al.*, 2017); while, Sulistyowati *et al.* (2010) found that the optimal level of yeast is 20 g/d in dairy cow; and DeVries and Chevaux (2014) stated that yeast supplementation could improve rumen metabolism and milk fat production.

Diet composition will also determine its digestibility, animal productivity, and quality of animal product. Rice straw, rice bran, and corn stover are produced in abundant quantity each year in Indonesia. Therefore, the usage of these forages are common, however, it is limited by season, rainy or dry. Wang *et al.* (2014) reported that rice straw as main forage produced lower nutrient digestibility, milk yield and milk quality.

This research was conducted to evaluate nutrients, fatty acid contents, *in vitro* characteristics (rumen liquor pH, *in vitro* dry matter and *in vitro* organic matter digestibility, N-NH₃ and total VFA productions) and *in vivo* nutrient digestibility of diet containing concentrate with inclusion of *Durio zibethinus* seed meal in lactating dairy cows.

Materials and Methods

Preparation of *D. zibethinus* seed meal

Durio zibethinus seed meal was prepared starting from collecting the seed, cleaning, washing, slicing thinly, drying under the sun until easily cracked, then grinding as meal. Slicing of *Durio* seed was carried out by using simple slicer made of wood with a blade on it, available in market. *Durio* seed was collected from some street vendors in Bengkulu city, originally was brought down from Bengkulu Tengah.

Preparation of *Durio* concentrate, yeast, curcuma, and treatments

Feed supplements, yeast was prepared by a procedure of Pusbangtepa (1981) and *Curcuma xanthorrhiza* Roxb meal was prepared by

washing, slicing, drying and grinding the roots of this curcuma. Curcuma served as antiprotozoa reported previously by Sulistyowati *et al.* (2014). The treatments were DC 35/12.5 (35% Rice bran+ 12.5% DSM), DC 27.5/20 (27.5% Rice bran+ 20% DSM), DC 20/27.5 (20% Rice bran+ 27.5% DSM) and DC 12.5/35 (12.5% Rice bran+ 35% DSM). Concentrate formulas containing *Durio* or named as *durio* concentrate (DC) seed meal are prepared as presented on Table 1.

Feed analysis

Dry matter (DM), crude protein (CP), crude fiber (CF), and ether extract (EE) were determined according to AOAC (2005). Nitrogen free extract (NFE) was calculated as 100% - (moisture + ash + CP + CF + EE). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined by the method of Goering and Van Soest (1970). Calcium (Ca) and Phosphor (P) were analyzed using Shimadzu AA - 7000 atomic absorbance spectrophotometer (AAS). Samples were prepared for analysis based on each procedure as can be accessed in Sulistyowati *et al.* (2014).

Analysis of fatty acid in *Durio* concentrate was detected using Shimadzu 2010 series gas chromatography (GC). The ratios of fatty acids were calculated based on the amount C in each fatty acid then grouped into C<10 as total short chain fatty acid (SCFA), C12-16 as medium chain fatty acid (MCFA), and C>16 as long chain fatty acid (LCFA), PUFA, ratio of unsaturated/saturated, ratio of PUFA/saturated and ratio of omega 6/omega 3 or n-6/n-3= (linoleate + arachidonate)/linoleate according to Schmidely *et al.* (2005).

In vitro procedure was conducted based on Tilley and Terry (1963), for N-NH₃, total VFA, *in vitro* dry matter digestibility (IVDMD), and *in vitro* organic matter digestibility (IVOMD) analysis. *In vivo* experiment was conducted to evaluate concentrates containing DSM in Latin square 4 x 4 arrangement in 4 lactating dairy cows. Nutrient intakes and nutrient digestibility were the parameters measured. Data were analyzed for variance, if any significant effects were found then further analyzed for differences using Duncan Multiple Range Test or DMRT (Lentner and Bishop, 1986). *In vivo* or apparent digestibility (%) was calculated based on the formula of nutrient

Table 1. Composition of concentrate containing *D. zibethinus* seed meal

Ingredients (%)	DC 35/12.5	DC 27.5/20	DC 20/27.5	DC 12.5/35
Rice bran	35.0	27.5	20.0	12.5
<i>Durio zibethinus</i> seed meal	12.5	20.0	27.5	35.0
Ground corn	25.0	25.0	25.0	25.0
Soybean meal	20.0	20.0	20.0	20.0
Palm oil	4.0	4.0	4.0	4.0
Mineral Mix	0.5	0.5	0.5	0.5
NaCl	0.5	0.5	0.5	0.5
Yeast	0.5	0.5	0.5	0.5
<i>Curcuma xanthorrhiza</i>	2.0	2.0	2.0	2.0
Total	100.0	100.0	100.0	100.0

Source: Modified from Sulistyowati *et al.* (2013).

intakes subtracted by nutrients in feces, divided by nutrient intakes, multiplied by 100.

Feed (45 kg/day forage, 24 kg of fresh soybean tofu by-product, 4 kg of on farm concentrate, and 2 kg/day DSM concentrates, according to the treatments with each ratio of rice straw and DSM) were provided throughout the experiment, the last seven days were assigned for feces collection and nutrient analyses. The forages contained of 50% king grass, 30% corn Stover, and 20% rice straw, as dry matter basis.

Result and Discussion

Nutrient composition of *Durio*- concentrate

Nutrient contents of concentrate containing *D. zibethinus* Murr seed meal is presented in Table 2. There were some inconsistencies in data, especially on crude protein and gross energy (GE) contents in the higher or lower ratio of rice bran and DSM. However, in general, increasing levels of DSM in concentrate in substitution of rice bran decreased the levels of crude protein, crude fiber, ether extract, Ca and P. Crude protein on concentrate with 20% of DSM was found the highest among treatments. This suggested that there was a contribution of the crude protein from DSM. This level of DSM is considered the optimal one. The higher the DSM, the crude protein decreased, however, they were higher than the lowest level of DSM, 12.5%.

On the other hand, organic matter and nitrogen free extract (NFE) were increased. Decreasing crude fiber, ether extract, Ca and P in the same pattern as the decreasing level of rice bran. This is due to the contents of crude fiber, ether extract, Ca and P in rice bran were higher than those in DSM. Calcium (Ca), 0.28-0.46% of DM, and phosphor (P), 0.13- 0.24% of DM, in these DSM- concentrates were much lower compared to those found in diets with different levels of protein in early lactation Holstein, were 0.81% Ca and 0.43% P as reported by Bahrami-Yekdangi *et al.* (2014).

Increasing organic matter and NFE were happened with the increasing level of DSM. The meal has high carbohydrate and sugar/starch

(62.9-70.7g and 47.9-56.4g/100g dry matter) (Charoenkiatkul *et al.*, 2016). Other ingredient that was in substitution with DSM was rice bran. Nutrient contents of the first quality rice bran are crude protein (11.9%), metabolic energy (2,200 kcal/kg), ether extract (12.1%), crude fiber (10.0%), phosphor (1.3%), and Calcium (0.1%) (Hartadi *et al.*, 1993). In this research result showed that substitution of rice bran with DSM in the level of 27.5% and 20%, respectively, showed higher crude protein (14.45%).

In vitro characteristics

Concentrate- DSM were then analyzed for *in vitro* characteristics (*in vitro* dry matter digestibility, IVDMD; *in vitro* organic matter digestibility, IVOMD). The *in vitro* characteristics of concentrate- DSM are presented on Table 3.

The treatments had no effects ($P>0.05$) on *in vitro* characteristics (*in vitro* dry matter digestibility, IVDMD; *in vitro* organic matter digestibility, IVOMD). In averages, the 35% of DSM in concentrate showed high IVDMD, IVOMD, N-NH₃, and total VFA. The mean pH value of rumen in this study was within the normal range. These pH levels were higher than those diets comprising of corn Stover and rice straw; while their N-NH₃ was higher and total VFA was lower as reported by Wang *et al.* (2014). Sung *et al.* (2007) reported that the rumen ideal pH for maintaining normal rumen metabolic processes is 6.0-7.0.

The averages of IVDMD, IVOMD, and total VFA in this research results were much higher than those found in *in vitro* characteristics of goat rumen fluid provided with concentrate supplemented with yeast and *C. xanthorrhiza* Roxb (Sulistyowati *et al.*, 2014). The total VFA in this current result was also much higher than that of in rice straw or corn stover which is about 85% (Wang *et al.*, 2014). However, the NH₃-N in this concentrate with DSM was slightly lower compared to the previous research. The ingredients of both concentrates were only different in carbohydrate sources (cassava by-products) as reported by Sulistyowati *et al.*, (2014); while, in this current formula contained DSM. This

Table 2. Nutrient contents of concentrate containing *D. zibethinus* seed meal

Variable	DC 35/12.5	DC 27.5/20	DC 20/27.5	DC 12.5/35
Dry matter (%)	91.64	91.63	91.47	91.52
Organic matter (%)	85.05	85.92	85.78	86.56
Ether extract (%)	10.45	10.82	10.09	9.87
Crude protein (%)	11.08	14.45	12.51	13.01
Crude fiber (%)	10.26	7.68	6.54	5.67
Nitrogen Free Extract	61.62	61.34	65.17	66.46
Ca (%)	0.46	0.29	0.35	0.28
P (%)	0.24	0.14	0.20	0.13
GE (cal/g)	4,391.39	4,278.20	4,525.69	4,130.99

Table 3. *In vitro* characteristics of concentrate containing *D. zibethinus* seed meal

Variable	DC 35/12.5	DC 27.5/20	DC 20/27.5	DC 12.5/35
pH rumen	6.88	6.84	6.80	6.74
<i>In vitro</i> dry matter digestibility (IVDMD) (%)	66.02	67.29	72.70	77.0
<i>In vitro</i> organic matter digestibility (IVOMD) (%)	65.15	65.99	71.22	75.15
N- NH ₃ (mM)	7.47	8.10	9.25	10.37
Total VFA (mM)	71.05	94.38	123.40	145.06

indicated that the Durio- concentrate had higher content of carbohydrate and organic matter digestibility, lower crude fiber content as well (Table 2), therefore, it yielded higher VFA (Table 3).

The NH₃-N levels and VFA production in this study were within the normal range. Sutardi (1977) stated that the range of optimal NH₃ and VFA production for the life of rumen microbes were 4-12 mM of NH₃ and 80-160 mM of VFA. This research data showed that the increasing level of DSM increased levels of NH₃, total VFA and digestibility of diets. The content of starch/amylose and amylopectin which is high enough in DSM caused the diet to be more easily digested and metabolized into VFA (volatile fatty acid). It is a raw material for further metabolism in ruminant livestock as precursor for formation of products such as milk and milk components (Seymour *et al.*, 2005). On the previous data, the VFA production of diet containing concentrate with 27.5% DSM supported the highest milk production. This indicated that this level of DSM was the optimal one for supporting milk production, even though the highest VFA was found in diet with the highest DSM (35%).

This indicated an indication that the DSM in concentrate to the highest level (35%) did have positive effects on IVDMD (77%), IVOMD (75.15%), N-NH₃ (10.37mM), and total VFA (156.06 mM). It presents more digestible nutrients that positively impact the life of rumen microbes and also on the animal who consumes concentrates composed of DSM.

Profile of fatty acids

Fatty acid content is one of nutrient that its quantity and quality affected by the feedstuffs ingested by the ruminant. Concentrate containing DSM in certain level in this research was expected to affect its fatty acid content. Fatty acid contents (short, medium, and long chain fatty acid) of concentrate – DSM are presented in Table 4. Averages of fat and fatty acid total found in

concentrate with 12.5% DSM were the highest, while, the lowest total fatty acid were shown in concentrate with 27.5% DSM. Palmitic acid, oleic acid, and linoleic acid are covered the most compared to other fatty acids.

Fatty acid classification profiles of DSM concentrate are presented on Table 5. Long chain fatty acid (LCFA) seemed decreasing with increasing DSM from 12.5% to 27.5%, however it started to increase back. Based on oil source, the LCFA of the diet that contained of 4% palm oil were lower, compared with other diet containing corn oil 2.8%, its LCFA is higher (77.8 g/100g of total FA) as reported by Boerman *et al.* (2014). It is suggested that the quantity of carbohydrate source and oil source will affect the quality and quantity of the fatty acids.

The results, especially P/SFA and UFA/SFA ratios are about in the same range of 2.51- 2.65 (Table 5). However, these ratios were lower compared to those in PUFA- concentrate that are about 4.06 (PUFA/SFA with *Curcuma xanthorrhiza* supplement in PUFA-concentrate) and 7.05 (UFA/SFA with *Curcuma xanthorrhiza* and yeast supplements in PUFA-concentrate) and about 13- 14 for n6/n3, within the same range in the concentrate with modified formula and ingredients as reported by Sulistyowati *et al.* (2015). These ratios were much higher compared to those in soybean oil (4.69- 6.12) as reported by Bouattour *et al.* (2008). The safe standard ratio of n6/n3 is about 5/1 – 10/1 according to WHO or World Health Organization (Bouattour *et al.*, 2008). These results suggested that DSM supplementation (20%) in concentrate seemed to be better in terms of higher PUFA total and unsaturated fatty acid, on the contrary, was lower in n6/n3.

Nutrient intakes and *in vivo* digestibility

Lactating dairy cows fed diet containing concentrate with different levels of 12.5- 35% of DSM showed the same amount of all nutrient intakes as shown in Table 6. Organic matter

Table 4. Fatty acid contents of concentrate containing *Durio zibethinus* seed meal

Items (g/ 100g of total fatty acid)	DC 35/12.5	DC 27.5/20	DC 20/27.5	DC 12.5/35
Fat (%)	11.44	10.25	10.84	10.37
C12:0, Lauric acid	0.07	0.07	0.05	0.07
C14:0, Myristic acid	0.34	0.35	0.26	0.32
C 15:0, Pentadecanoic acid	0.02	0.02	0.02	0.02
C16:0, Palmitic acid	19.68	19.95	18.39	19.89
C16:1, Palmitoleic acid	0.11	0.11	0.08	0.10
C17:0, Heptadecanoic acid	0.07	0.07	0.06	0.07
C17:1, Cis-10- Heptadecanoic acid	0.02	0.03	0.02	0.03
C18:0, Stearic acid	2.54	2.51	2.33	2.60
C18:1n9t, Elaidic acid	0.04	0.03	0.03	0.03
C18:1n9c, Oleic acid	28.87	28.45	27.01	27.60
C18:2n6c, Linoleic acid	30.59	29.56	27.77	29.05
C18:3n3, Linolenic acid	2.12	2.20	1.87	2.09
C20:0, Arachidic acid	0.26	0.25	0.20	0.24
C20:2, Cis-11,14-Eicosadienoic acid	0.06	0.05	0.07	0.10
C21:3, Heneicosanoic acid	-	-	0.02	0.04
C22:0, Behenic acid	0.18	0.19	0.16	0.16
C23:0, Tricosanoic acid	0.02	0.02	0.02	0.03
C24:0, Lignoceric acid	0.16	0.16	0.10	0.10
C22:6n3, Cis-4,7,10,13,16,19-docosahexaenoic acid,	-	0.03	0.04	0.06
Fatty acid total	85.16	84.04	78.50	82.58

Table 5. Classification of fatty acid contents of concentrate containing *Durio zibethinus* seed meal

Items (g/100 g of total FA)	DC 35/12.5	DC 27.5/20	DC 20/27.5	DC 12.5/35
Short CFA (C4-C10) total	nd	nd	nd	nd
MCFA (C12- C16) total	20.22	20.5	18.8	20.4
LCFA (C>C16) total	64.93	63.55	59.7	62.2
MUFA total	0.17	0.17	0.13	0.16
PUFA (P) total	61.64	60.29	56.78	58.94
Saturated fatty acid (SFA) total	23.34	23.59	21.59	23.5
Unsaturated fatty acid (UFA) total	61.81	60.46	56.91	59.1
Ratio P/SFA	2.64	2.56	2.63	2.51
Ratio UFA/SFA	2.65	2.56	2.64	2.51
n6/n3	14.55	13.55	14.96	14.01

Table 6. Nutrient intakes of diet containing concentrate with *Durio zibethinus* seed meal

Variable	DC 35/12.5	DC 27.5/20	DC 20/27.5	DC 12.5/35
Nutrient intake, kg/d				
Dry matter	22.50	22.32	22.41	22.54
Organic matter	15.71	15.63	15.68	15.77
Crude protein	2.97	3.02	2.90	3.01
Ether extract	0.79	0.79	0.78	0.78
Crude fiber	4.75	4.65	4.66	4.67
N- free extract	7.11	7.07	7.16	7.21

Table 7. Nutrient apparent digestibilities of diet containing concentrate with *Durio zibethinus* seed meal

Variable	DC 35/12.5	DC 27.5/20	DC 20/27.5	DC 12.5/35
Apparent digestibility, %				
Dry matter	76.06	75.59	76.41	74.74
Organic matter	77.46	77.24	78.66	76.37
Crude protein	76.76	76.42	77.54	75.55
Ether extract	89.63 ^a	90.88 ^b	88.81 ^a	90.90 ^b
Crude fiber	76.07	75.47	76.22	75.72
N- free extract	71.45	70.96	74.33	69.93

^{a,b} Means in the same row were different significantly (P<0.05)

intakes in this research (with king grass, corn stover, and rice straw, besides DSM concentrate) were about 15.63- 15.77 kg/d which was slightly lower than it was with rice straw and corn stover (Wang *et al.*, 2014). Arndt *et al.* (2014) reported that dry matter digestibility of diet with corn grain and high forage was 65.2% while its organic matter digestibility was 67.5% and crude protein digestibility was 64.0%. Compared to these results, supplementation of 27.5% Durio meal showed the highest digestibility of dry matter, organic matter, and crude protein, which were 76.41, 78.66, and 77.54%, respectively; while ether extract digestibility significantly (P<0.05) lower than that of with 20% of DSM and 35% of DSM. These differences may have been due to high forage level that was applied in other diet.

Supposedly, this high level of Durio meal in concentrate would give high digestibility as its energy source is much higher in soluble carbohydrate then easily metabolized. However, it did happen in the opposite way. This might be due to the process of preparation of the DSM in which the outer skin of the seed was not peeled off. Therefore, it caused high content of more structural carbohydrate that eventually decreased the solubility and digestibility of concentrate containing highest Durio (35%) seed meal as expressed in the low digestibility in dry matter, organic matter and crude protein (Table 7).

On the other hand, concentrate containing 27.5% DSM seemed to have high averages on apparent digestibility of dry matter, organic matter, crude protein, crude fiber, and N-free extract. The digestibility is affected by energy density of the feed, especially determined in organic matter. Dry

matter intake in all levels of DSM showed 19.6% higher than those found in cows fed corn Stover and rice straw; while, their other nutrient digestibilities were lower (Wang *et al.*, 2014). This dry matter intake was much higher than it is suggested by NRC (2001). However, the DSM-concentrate containing more readily carbohydrate in concentrate of the diets, causing higher nutrient digestibility up to certain level, that was 27.5% of DSM. Other research showed higher dry matter intake in high producing cows fed diet containing palmitate with lower digestibility (Rico *et al.*, 2014). This can be described by Jenkin (1993) and Maia *et al.* (2007) that unsaturated fatty acid can have toxic effects on ruminal bacteria and decrease ruminal digestibility.

Conclusions

The 20% of DSM supplemented in concentrate improved nutrient contents of crude protein, ether extract, medium chain fatty acid, long chain fatty acid, polyunsaturated fatty acid, unsaturated fatty acid, and n6/n3. The 35% DSM in concentrate showed better performance of *in vitro* dry matter digestibility, organic matter digestibility, N-NH₃, and total VFA. In *in vivo* digestibility evaluation showed no differences in nutrient intakes. Nutrient digestibility were found higher in diet with concentrate containing 27.5% of DSM. Therefore, there were three different optimal levels, 20% of DSM (for nutrient and fatty acid content), 27.5% of DSM (for nutrient digestibility), and 35% of DSM (for *in vitro* characteristics). Supported by previous data of milk production, it was concluded that the 27.5%

of DSM was suggested to be applied in concentrate of diet for dairy cows.

Acknowledgment

This research was part supported by a grant from Kemenristek-DIKTI through DRPM under the scheme of Stranas 2018 with contract no: 052/SP2H/LT/DRPM/2018. We are very thankful for the grant. Sincere appreciations are truly delivered to Prof. Dr. Ir. Edy Kurnianto, MS., MAgr and Prof. Dr. Ir. Dewi Apri Astuti, MS for reviewing and supporting this manuscript to be published.

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