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Evaluation of Mineral Contents in Milk of Dairy Cattle Fed Elephant Grass Planted at Ex-Coal Mining Land

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ABSTRACT

The growth of several types of grass in the area of mine re-vegetation is rapidly providing an opportunity to be used as forages despite the possibility of heavy metal contamination. The purpose of the study was to evaluate the effect of treatment of different levels of elephant grass (EG) (*Pennisetum purpureum)* planted at ex-coal mining reclamation on milk productivity and mineral contents of dairy cattle. This study used four lactating (second lactation) dairy cattle using Latin Square Design of 4 (treatments) x 4 (replications). Each period was 21 days consisted of 14 days of *preliminary* for feed adaptation and 7 days for observation. Treatments were P0 (0% ex-coal mining (EEG)+60% Farmer's EG (FEG)+40% Concentrate), P1 (15% EEG+45% FEG+40% Concentrate), P2 (30% EEG+30% FEG+40% Concentrate), and P3 (60% EEG+0% FEG+40% Concentrate). The study observed variables of feed intake and efficiencies, milk production, milk quality, and mineral contents of milk. Results showed that EEG treatment at different levels did not affect fresh and dry matter intake. The highest fresh and dry matter intake was found in P0 treatment. There was no significant difference in giving EG planted at the ex-coal mining and the farmer's land on the milk production and milk quality. The only significant differences ($p<0.05$) were found in mineral Fe and Mg contents of milk. The study concluded that giving EG planted at the ex-coal mining area until 60% of forage affected Fe and Mg contents of milk, but they are still in the safe limit.

***Keywords:** ex-coal mining; elephant grass; heavy metal; milk production and quality*

INTRODUCTION

Total coal productions in Indonesia are 419 million tons, while total coal productions in East Kalimantan are 236.6 million tons (Statistics Indonesia, 2018). National Standardization Agency of Indonesia (1998) stated that coal in East Kalimantan belongs to the moderate geological group, which is characterized by the slope of the layers and moderate variations in lateral density and the development of branching of coal seams. However, the distribution is still up to hundreds of meters. Coal reserves can be found beneath or above the surface of the earth, in which surface coal mining processes inevitably cause mine soils to degrade (Feng *et al.,* 2019). Mining activities contain sulfide minerals such as coal, which triggers acid formation. The excavation process causes the uplifting of sulfide material to the surface. Consequently, oxidation occurs, resulting in a drastic decrease in soil humidity (pH).

The mining industry area is often associated with the possibility of heavy metal contamination. The growth of several types of grass in the mine re-vegetation area quickly covers the land, and these grasses are useful for foraging and livestock grazing. The use of forage planted on ex-mining land, however, must be assessed as it may contain heavy metals. Hence, before its usage for food crops or forages, the ex-mining land reclamation must be carried out (Licina *et al.,* 2017). The number of studies found that heavy metal is a general term that applies to groups of metals and metalloids of metals with a five or more density such as Mn, Cu, Zn, As, Se, Sb. These metals can cause human health problems, for example, cancer. It is because those metals can induce oxidative stress, as well as change the function of protein and DNA due to mining, smelting, industrial, agricultural, and sewage waste (Lim *et al.,* 2019; Kim *et al.,* 2019). Consumption of food containing heavy metals for human is a significant factor in exposure of heavy metals that causes detrimental health problems (Soekomo *et al.,* 2011; Ali & Malik, 2011; Donaldson *et al.,* 2010).

Based on the results of a preliminary analysis at the laboratory, mineral contents of elephant grass (EG) planted in Integrated Dairy Farming (IDF) of KPC's ex coal-mining land was 110; 200; 0.45 and 64.19 ppm of Mg; Fe; Pb and Mn, respectively. The feed given to the dairy cattle at IDF was EG planted at ex-coal mining land. Therefore, it becomes our concern in analyzing those minerals content in milk. The mineral contents in feed affect mineral contents in milk. This study evaluated the mineral contents of milk in dairy cattle fed with EG planted at the ex-coal mining land. The study will provide a valuable information on healthy milk for humans; especially, the milk comes from dairy cattle raised in the area of ex-coal mining land. There is a lack of studies to assess the potency of ex-coal mining land for livestock. This study will determine the possibility of this type of land used for forage-fed by livestock, notably, for dairy cattle, including the impact of this forage to milk quality in terms of heavy metals content.

MATERIALS AND METHODS

This research was conducted for 5 months (July-November 2018) in integrated dairy farming (IDF) at PT Kaltim Prima Coal (KPC) Sangatta, East Kalimantan, Indonesia. This study used four FH dairy cattle (± 5 years of age; average body weight of 392.5±9.57 kg) from Batu, Malang, Indonesia, at the second lactation period. Latin square was used for the experimental feeds treatments, namely P0 (0% ex-coal mining (0%EEG + 60% FEG + 40% Concentrate), P1 (15% EEG + 45% FEG 40% Concentrate), P2 (30% EEG + 30% FEG + 40% Concentrate), and P3 (60% EEG + 0% FEG + 40% Concentrate). Manure that enters the ditch automatically flows into the compost digester. The manure was then processed in the digester that will produce liquid fertilizer and biogas. The resulting liquid fertilizer was then distributed to the grass garden that was used for soil fertility and grass growth itself (Table 2). The grass was calculated based on nutrient contents of basal feeds, as shown in Table 1, in accordance with the nutrients required by lactating dairy cattle with a bodyweight of ± 400 kg, as mentioned in NRC (2001) for CP and TDN are 15%-16% and 67%-70%, respectively.

In Vivo Trial

The experiment used 4 dairy cattle (average bodyweight of 392.5±9.57 kg). The dairy cattle were treated with worm medicine and vitamin B before the experiment. The trial period was 3 months, divided by 4 periods each with of 21 days. Each period consisted of 14 days of preliminary period intended to eliminate the effect of the previous feed and adaptation to a new feed followed by a period for observation for 7 days. The experimental feed treatments are presented in Table 2. The amount of rations given per day to the experimental animals was based on dry matter (DM) required for their level of production being 3.5% of the live weight. The one day diet was divided into two parts and given twice a day (08.00 a.m. and 03.00 p.m.). Drinking water from the artificial river with pH 6.8 was provided *ad libitum*.

The experimental cattle were milked twice a day, and the total milk production was recorded. The quality of milk was measured every day during the observation period using *Lactoscan* serial 14-9224 supply 12V DC 50W made in Bulgaria. The sub-sample for mineral analysis was taken at the end of the study period. Fresh milk samples were taken from morning milking in a volume of 500 mL from each dairy cattle. Then the milk sample was put in a 100 mL polyethylene plastic sterile and stored at 4°C. Sampling from the research location to the laboratory was carried out by storing samples

Table 1. Nutrients content of basal feed used in the experiment

Nutrients	Feed Pesat I (Concentrate)	Elephant grass	
		Farmer	Ex-coal mining
Dry matter (%)[1]	100.00	100.00	100.00
Ash (%)[1]	6.18	5.63	8.87
Crude protein (%)[1]	11.56	8.76	11.32
Extract ether (%)[1]	9.67	2.66	2.47
Crude fiber (%)[1]	15.63	33.58	29.43
NFE (%)[1]	44.92	17.36	16.43
TDN (%)[2]	70.88	38.51	36.66
Ca (%)[1]	0.97	0.25	0.27
P (%)[1]	0.62	0.14	0.47
Mg (ppm)[3]	5056.092	2430.102	2766.892
Mn (ppm)[3]	123.224	671.99	226.97
Fe (ppm)[3]	160.03	54.99	168.621
Pb (ppm)[3]	0.005	3.636	6.251
As (ppm)[3]	0.002	0.002	0.002
Hg (ppm)[3]	0.132	0.012	0.416

Note: [1]Laboratory Animal Feed Technology Pajajaran University; [2]Formula TDN (Wardeh, 1981); TDN (%DM) energy sources = 40.2625 + 0.19699CP%) + 0.4228 (NFE%) + 1.1903 (EE%) - 0.1379(CF%). TDN (%DM); forage= 1.6899+1.3844 (CP%) – 0.8279 (EE) + 0.3673 (CF%) + 0.7526 (NFE%); [3]Dairy Farming Nutrition Laboratory at IPB University.

Table 2. Nutrients content of experimental feed treatments

Nutrients	Treatments			
	P0	P1	P2	P3
Dry matter (%)	48.5	48.4	48.2	47.8
Ash (%)	5.85	6.34	6.82	7.79
Crude protein (%)	9.88	10.26	10.65	11.42
Extract ether (%)	5.46	5.44	5.41	5.35
Crude fiber (%)	26.40	25.78	25.16	23.91
TDN (%)	51	51	51	50
Ca (%)	0.538	0.541	0.544	0.550
P (%)	0.332	0.382	0.431	0.530
Mg (ppm)	3480.498	3531.02	3580	3680
Mn (ppm)	90	113.57	140	190
Fe (ppm)	97.006	114.05	131	165
Pb (ppm)	2.1836	2.58	2.981	3.7526
As (ppm)	0.002	0.002	0.002	0.002
Hg (ppm)	0.06	0.1206	0.1812	0.3024
Feed composition (%)				
EEG	0	15	30	60
FEG	60	45	30	0
Concentrate	40	40	40	40

Note: EEG= ex-coal mining elephant grass; FEG= farmer elephant grass; P0= 0% EEG + 60% FEG + 40% Concentrate; P1= 15% EEG + 45% FEG + 40% Concentrate; P2= 30% EEG + 30% FEG + 40% Concentrate; P3= 60% EEG + 0% FEG + 40% Concentrate in a cool box containing dry ice and being taken to the dairy farming nutrition laboratory at IPB University to analyze the mineral contents.

Variables Measurement

The measurement of feed consumption was carried out by calculating the difference in the amount of feed given with the excess feed remaining every day during the study period. Consumption of feed (forage and concentrate) and DM intake were recorded. Measurements were carried out every 24 hours for 7 days in a period.

Milk production was calculated based on fat corrected milk (FCM) (NRC 2001). Technical efficiency (TE) was calculated by using the value based on milk production per unit of dry matter intake. In addition, the economic efficiency (EE) value was measured based on the profit from milk production on the feed cost used. The feed cost (1liter^{-1}) of milk was estimated from the total feed cost used to produce every one liter of milk. Income over-feed cost of Milk (IOFC of Milk) (Price head^{-1} day^{-1}) was calculated by reducing the income from milk sale by the cost of feed (Buza *et al.*, 2014).

The study evaluated milk quality based on the composition of protein, fat, solid non-fat (SNF), dry milk ingredients, and lactose was tested by using *Lactoscan* (serial 14-9224 supply 12V DC 50W made in Bulgaria). Mineral contents in milk such as Mg, As, Mn, Fe, Pb, Hg were also evaluated by using a *spectrophotometer* (IPB 2014).

Data Analysis

The data were analyzed by using analysis of variance (ANOVA) and any significant difference among treatments were tested by Duncan test (Steel & Torrie, 1995) using software SPSS 16, at the level 5% ($p<0.05$).

RESULTS

Feed Consumption

Feeding lactating cattle with EEG did not affect the consumption of fresh feed and nutrient (Table 3). Mineral consumption is calculated based on the usage of DM multiplied by the mineral content of the treatment ration. According to statistical analysis, offering the EEG with different levels did not alter the consumptions of Pb, Fe, Mg, Mn, As, and Hg.

Milk Production, Technical Efficiency, Economic Efficiency, and IOFC

Data on milk production, TE, EE, and IOFC of experimental dairy cattle are presented in Table 4. The statistical analysis showed that feeding lactating cattle with EG from ex-coal mining land did not significantly affect the 4% FCM milk production. Based on the statistical analysis, there was no significant difference in the average values of the efficiency of technical ration in each treatment.

Quality of Milk

The results of the statistical analysis revealed that the feeding of the experimental cattle with EEG did not significantly affect the quality of milk, namely protein, fat, SNF, and lactose contents (Table 5). The average milk protein contents in treatments were similar for all the treatment groups.

Mineral Contents in Milk

Statistical analysis showed that experimental lactating cattle with EG from ex-coal mining land significantly

Table 3. The average consumption of fresh feed (kg head^{-1} day^{-1}) and nutrients in % dry matter (g head^{-1} day^{-1}) in dairy cattle

Consumption	Treatments			
	P0	P1	P2	P3
Fresh (kg head^{-1} day^{-1})				
Elephant grass	38.25±0.50	38.25±0.95	37.00±3.36	37.00±2.82
Concentrate	9.25±0.95	9.50±0.57	9.50±0.57	9.50±0.57
Dry matter (g head^{-1} day^{-1})				
Dry matter	16937.47±545.60	16125.14±1303.28	16746.73±573.47	16429.61±492.20
Crude protein	1717.64±60.78^{b}	1700.49±139.16^{b}	1858.16±27.42^{a}	1880.165±55.85^{a}
Crude fiber	4898.16±615.05	4045.58±188.15	4076.57±37.08	3666.09±144.09
Extract ether	1036.16±47.17	957.26±118.82^{b}	1037.96±21.56^{a}	1015.57±28.11
TDN	9068.09±359.09	8547.69±888.54	9142.57±224.02	9228.39±320.
Pb (ppm)	0.9532±0.01	1.1347±0.02	1.2621±0.09	1.6086±0.11
Fe (ppm)	27.7791±0.87	34.2293±2.19	42.1845±2.03	56.9388±2.75
Mg (ppm)	1059.193±29.58	1048.2944±68.96	1097.018±46.98	1140.192±43.67
Mn (ppm)	186.3859±2.59	157.3223±4.09	125.9111±8.65	68.8321±3.81
As (ppm)	0.0069±0.00	0.006±0.00	0.0068±0.00	0.0068±0.00
Hg (ppm)	0.1200±0.00	0.930±0.00	0.0664±0.00	0.0142±0.00

Note: TDN= Total digestible nutrient; EEG= ex-coal mining elephant grass; FEG= farmer elephant grass; P0= 0% EEG +60% FEG + 40% Concentrate; P1= 15% EEG + 45% FEG + 40% Concentrate; P2= 30% EEG + 30% FEG + 40% Concentrate; P3= 60% EEG + 0% FEG + 40% Concentrate. Means in the same row with different superscripts differ significantly ($p<0.05$).

influenced the Fe and Mg contents of the milk produced (Table 6). The differences were found among the treatment groups. However, the statistical differences were not significant for Pb, Mn, As, and Hg contents. The lowest average Fe content was found in P0 group, and the highest was found in P3 group. The average Pb contents in this study for treatment P0-P2 was 0.005 ppm, and P3 was 0.0063 ppm. The average As and Hg contents in this study for treatments were 0.003 and 0.005 ppm, respectively.

DISCUSSION

Feed Consumption

In the present study, the consumption of nutrients in dairy cattle was not affected by feeding with EEG. Dry matter intake (DMI) in the study was similar to the result reported by Olijhoek *et al.* (2018) that DMI at Freisien Holstein and Jersey cattle were 21100±3000 g day^{-1} and 17400±2190 g day^{-1}, respectively. Trace mineral (TM) content of EEG was higher than FEG (Table 1). The result agreed with Miller *et al.* (2019) reporting that TM sources affected DMI. Additionally, DMI is influenced by the source roughage with its total mixed fiber (Maneerat *et al.*, 2013) and forage particle size (Reynolds *et al.*, 2018).

As a result, feeding the experimental dairy cattle with EEG did not affect the consumption of CP. Crude protein intake is influenced by CP composition (Dickhoefer *et al.*, 2018). Consumption of CP was influenced by the increasing level of protein in the feed. Therefore DMI, milk component, and yield, and feed efficiency will be increased with the increasing level of CP in the feed (Ramin *et al.*, 2019; Zanton, 2016). The increased level of CP in the feed is related to the N content of the EG grown on the ex-coal mining land, since the application of manure compost on the land resulting in the increasing N content of grass. Nitrogen has a positive correlation with protein that low nitrogen and low phosphorus affect protein content and protein phosphorus, and low phosphorus had smaller influence than low N treatment (Toth *et al.*, 2020; Andrianasolo *et al.*, 2016).

Feeding dairy cattle with EEG did not affect the intake of crude fiber (CF). Dry matter, organic matter, NDF, ADF, and CP digestibilities affected dairy cattle

Table 4. Milk production, technical efficiency, economic efficiency, and income feed over cost (IOFC)

Variables	Treatments			
	P0	P1	P2	P3
Milk production FCM 4%	6.51±0.83	6.79 ± 0.96	6.68 ± 1.07	7.67 ± 0.90
Technical efficiency	0.18±0.02	0.19±0.02	0.19±0.02	0.22±0.03
Economic efficiency	1.23±0.07	1.30±0.30	1.34±0.22	1.58±0.23
Feed cost per liter of milk	11477.34±1736.857	10686.1±2036.29	10318.47±1768.51	8265.07±971.92
IOFC of milk (Rp e^{-1} hr^{-1})	13423.08±4158.65	21666.67±21320.9	22946.43±15149.54	35803.57±14601.63

Note: EEG= ex-coal mining elephant grass; FEG= farmer elephant grass; P0= 0% EEG +60% FEG + 40% Concentrate; P1= 15% EEG + 45% FEG + 40% Concentrate; P2= 30% EEG + 30% FEG + 40% Concentrate; P3= 60% EEG + 0% FEG + 40% Concentrate.

Table 5. Data on the quality of milk taken from dairy cattle fed the experimental feed

Variables	Treatments			
	P0	P1	P2	P3
Protein (%)	2.69±0.17	2.74±0.17	2.76±0.06	2.80±0.13
Fat (%)	4.95±0.81	4.89±0.71	4.93±0.48	5.24±0.70
SNF (%)	7.65±0.38	7.49±0.47	7.53±0.18	7.40±0.28
Lactose (%)	4.19±0.20	4.11±0.26	4.14±0.10	4.06±0.15

Note: EEG= ex-coal mining elephant grass; FEG= farmer elephant grass; P0= 0% EEG +60% FEG + 40% Concentrate; P1= 15% EEG + 45% FEG + 40% Concentrate; P2= 30% EEG + 30% FEG + 40% Concentrate; P3= 60% EEG + 0% FEG + 40% Concentrate.

Table 6. Data on mineral contents of milk produced from dairy cattle fed experimental feeds

Mineral (ppm)	Treatments				Threshold
	P0	P1	P2	P3	
Pb	0.005±0	0.005±0	0.005±0	0.0063±0.002	0.02*)
Fe	0.160±0.001^{a}	0.0385±0.009^{c}	0.0298±0.008^{bc}	0.126±0.006^{b}	1.00**)
Mg	99.77±28.32^{a}	105.51±10.99^{ab}	107.48±31.95^{b}	109.51±14.37^{b}	150**)
Mn	0.0105±0.001	0.00807±0.0006	0.0261±0.002	0.0533±0.007	0.082***)
As	0.002±0	0.0035±0.001	0.002±0	0.0041±0.0001	0.1*)
Hg	0.007±0.001	0.005±0	0.005±0	0.006±0.002	0.03*)

Note: *)BSN (2011); **)NRC (2001); ***)FAO (2002); ****)Ogabiela *et al*. (2011); EEG= ex-coal mining elephant grass; FEG= farmer elephant grass; P0= 0% EEG +60% FEG + 40% Concentrate; P1= 15% EEG + 45% FEG + 40% Concentratee; P2= 30% EEG + 30% FEG + 40% Concentrate; P3= 60% EEG + 0% FEG + 40% Concentrate. Means in the same row with different superscripts differ significantly ($p<0.05$).

nutrient digestibility (Fu *et al.*, 2019). The result of CP intake found in the present study was similar to the data of Sousa *et al.* (2017) that showed 1650 up to 2007,5 g h^{-1} day^{-1}. In addition, intake, such as total digestible nutrients, will decrease the nutritive value of diets for lactating dairy cattle (Cunha *et al.*, 2013).

The current study found that fat intake was not affected by feeding lactating dairy cattle with EEG. This result agreed with Hess *et al.* (2019) who reported that the grain component of the basal diet did not affect milk yield of dietary fat supplements. Admittance levels and types of dietary energy sources, such as starch and fat, affected plasma metabolite profiles, milk production, and fertility of dairy cattle (Useni *et al.*, 2018). In addition, feeding fats have a positive effect on fertility and a tendency to increase production when fed during the transition period (Rodney *et al.*, 2015). However, fat consumption increased milk production and milk fat but decreased protein yield (Rabiee *et al.* (2012).

The study revealed that TDN had not been influenced by giving dairy cattle with EEG. This result agreed with the reults reported by Marchi *et al.* (2013) that pelleting and lignosulfonate treatments influenced diet intake and nutrient digestibility but were not affected by ground seeds. Additionally, TDN intake influenced CF, which is a source of roughage used in the diet was corn silage similar to diets based on a combination of two roughages (Alessio *et al.*, 2018).

Consumption of TM was not affected by feeding the experimental dairy cattle with EEG. Diets should contain Mn at the levels of 30 ppm at lactating and 50 ppm at dry cows (Safdar & Kor, 2014). Additionally, a high K intake is the most important dietary factor that inhibits Mg absorption, which entails the risk of Mg deficiency, so supplementation of Magnesium oxide (MgO) considerably applied in practice and recommended to increase Mg in the diet (Schonewille 2013).

Milk Production, Technical Efficiency, Economic Efficiency, and IOFC

Milk product of 4% FCM in this study was lower than that reported by Maneerat (2013), i.e., 12.36±2.56 to 15.10±2.09, which resulted from the total mixed fiber. Maneerat (2013) highlighted that EG was better than pineapple silage but without significant effect on milk composition. Milk yield was not affected by fiber content (Gaafar *et al.* 2010), but it was affected by dietary protein and starch (Sucak *et al.*, 2017). This study indicated that TE of milk production was low (Al-Sharafat, 2013). Economic efficiency (EE) in this study was similar to the results reported by Unakitan & Kumbar (2019), i.e., 1.22. In addition, milk yield as a model input in intake predictions can be substantially affected by current dietary factors (Krizsan *et al.*, 2014).

Furthermore, dietary forage and CP content will have positive economic and environmental impacts on dairy production under tropical conditions (Corea *et al.*, 2017). EEG did not affect feed cost per liter of milk. Productivity, which plays a dominant role in a dairy farm, the cost of milk production could be reduced substantially if feeding practices and management of dairy animals are on scientific lines (Dubey *et al.*, 2017. Optimal ration formulation rather than least-cost strategies may be vital in increasing milk yield and IOFC (Buza *et al.*, 2014). Finally, the stochastic approach can be improved by using more inputs at the dairy farm level and considering the actual cost to measure profitability (Atzori *et al.*, 2013).

Quality of Milk

The result of the protein content of milk recommended with BSN (2011) reported that the minimum protein content is 2.75%. Nutrition affects the quality and component of milk (Tyasi *et al.*, 2015). Moreover, milk production efficiency affected milk quality because of the efficiency of nutrient absorbtion was affected by dry matter intake (Martono *et al.*, 2016) as well as improved forages qualities have the potential to increase milk production and milk quality (Mwendia *et al.*, 2018). Particularly, iron contamination in bovine drinking or milk processing plant can change milk protein composition and oxidation in the final milk product, which decreases the quality and nutritional value of milk (Wang *et al.*, 2016).

The result agreed with BSN (2011) that requires the minimum content of fat is 3.0%. Additionally, improving milk fatty acid profiles and milk production can be the combined effects of corn stalk and other roughage (Liu *et al.*, 2016). The high level of milk fat is affected by feed consumption, primarily forage consumption as a source of fiber. Diet is a source of nutrients for an animal that plays a role in the production and composition of milk. Milk fat levels can be affected by several factors, including the type of dairy cattle, age, lactation month, milking interval, environmental conditions, and diet consumption. Milk in tropical dairy herds consisted of fat, protein, lactose, and SNF 3.46; 3.39; 4.73, and 8.66 (Patricia *et al.*, 2015).

In the present study, SNF meets the standard requirement of national standard (BSN, 2011), which requires the minimum content of 7.8%. In the other study, SNF was affected by many factors such as the decrease of milk fat, the rise of milk dry matter and milk specific gravity **(**Adhani *et al.*, 2012**).** Also, increasing dietary fiber content influenced the content of fat, protein, lactose, SNF, and total solid (TS) significantly (Gaafar *et al.*, 2010).

In the present study, feeding the experimental dairy cattle with EEG did not affect the lactose content of milk. The result was similar to a previous study (Nichols *et al.*, 2018) which reported that produced energy from fat and protein was the same when supplemented at isoenergetic levels. Furthermore, the formation of lactose was more affected by propionic acid, which comes from a concentrate or high-energy feed, and the higher concentrate content will decrease milk fat (Utami *et al.*, 2014; Ramli *et al.*, 2009). Therefore, lactose contributes to the energy value of milk and it is an essential ingredient for the food and pharmaceutical industries (Costa *et al.*, 2019).

Mineral Contents of Milk

The present study showed that dairy cattle fed with EEG affected Fe and Mg contents in milk. The result of this study was lower compared to a report by Manuelian *et al.* (2018) that parity, stage of lactation, and breed were essential contributors to milk mineral variation with Mg. Also, the highest average of Mg content was ration with 60% elephant grass from ex-coal mining land. However, Mg content in milk was still below the average, according to NRC (2001), which was 150 ppm. Therefore, the higher the level of EEG, the higher the Mg content in milk. Retention and absorptions of Mg in dairy cattle relate to their requirements. Mg in feed rations must always be available. It is related to Mg retention and absorption in dairy cows. The age of dairy cattle had a significant effect on the average Ca and Mg contents of milk throughout lactation (Nogalska *et al.*, 2017).

In the present study, feeding of experimental dairy cows with EEG significantly ($p<0.05$) affected Fe contents in milk. The result was lower compared to previous reports (Davidov *et al.*, 2019; NRC, 2001). The mineral contents of milk were affected by some factors such as soil, air, water, and environments. Moreover, HM has a strong relationship in soil, blood, and milk (Tahir *et al.*, 2017). Also, Fe was an essential micromineral and needed in several biological processes, but if excessive, it will produce free radicals and attack sensitive tissues. The Fe originated from drinking water or iron supplements that cause oxidation, affect the composition and stability of milk protein, as well as the final milk quality (Wang *et al.*, 2014; Ganz & Nemeth, 2006). Several studies revealed that feed with Fe would increase competition for the absorption of Mn and Cu in the small intestine because these minerals were antagonistic to ruminants (Hansen & Spears, 2009). The Fe was present in excessive amounts of feed, and it would be toxic because Fe would induce the production of Fe binding proteins in erythrocytes called ferritin (Goft *et al.*, 2018).

The study revealed that mercury (Hg) was not found in the milk of dairy cattle fed with EEG. According to BSN (2011), the maximum toleration of Hg on milk is 0.03 ppm. Also, Hg was not found in any area with a high probability of being highly contaminated due to a mining zone (Montana *et al.* 2019. Therefore, metal concentration in dairy milk tends to increase with the increase in lactating age, which could be the cause of metals' ability to bio-accumulate (Kabir *et al.*, 2017).

The study reported that arsenic (As) content of milk of dairy cattle was not affected by feeding with EEG. Also, it was reported that As content of milk in the area contaminated with mining land were 0.0184±0.0068 and 0.1664±0.0423 ppm in liquid and lyophilized milk, respectively (Montana *et al.*, 2019). Besides, lactating livestock exposed to high levels of toxic metals (Pb, Sn, Hg, Cd, Zn, As, Cu, and Fe) accumulate these metals in their milk, thus posing a severe health risk for consumers (Ogut *et al.*, 2016)

Mangan (Mn) is one of the essential minerals for dairy cattle. In the present study giving elephant grass from ex-coal mining land did not affect Mn content of milk. In the other study, the mineral profile in milk seems possible for many minerals, but it likely depends on genetics, environmental, and management factors in variable proportions according to the mineral considered (Stocco *et al.*, 2019). The study reported that Pb content in fresh milk in a permittable concentration or a maximum of 0.02 ppm, according to BSN (2009). The contamination of milk came from multiple sources. There was a positive correlation between Pb and As concentrations in milk and water. Also, there was a positive correlation between Cr concentration and Cd concentration in milk and soil (Zhou *et al.*, 2019). Gravert (1987) stated that HM in fresh milk of dairy cattle was absorbed from contaminated feed for around 5%-10% and then excreted through milk. Pb consumption in excessive amounts will be damage to the nervous system (BSN, 2009).

CONCLUSION

Treatment of elephant grass from ex-coal mining in Integrated Dairy Farming (IDF) increases the content of Fe and Mg minerals in milk. However, the ranges of Fe and Mg concentrations are still below the allowable threshold. Minerals of Pb, Fe, Mg, Mn, As, and Hg in elephant grass of ex-coal mining were higher than the farmer's land elephant grass. However, those minerals grass of ex-coal mining were still safe to be consumed by dairy cattle to produce milk with allowable mineral contents of Pb, Fe, Mg, Mn, As, and Hg in dairy milk.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with the other people or organizations related to the material discussed in the manuscript.

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