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ANALYSIS OF RUMEN DEGRADATION CHARACTERISTICS OF FORAGE CRUDE PROTEIN IN GOAT

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ABSTRACT: The quality of feed given to ruminants can be determined from the degradation of nutrient content in the rumen. This study aimed to determine the pattern of forage degradation and the characteristics degradation of crude protein in the rumen using the *in sacco* method. The study used 4 fistulae kacang goats with an average body weight of 14.57 kg. The forage used consisted of R1: elephant grass (*Pennisetum purpureum*), R2: mini elephant grass (*Pennisetum purpureum cv. Mott*), R3: guinea grass (*Panicum maximum*), and R4: signal grass (*Bracharia decumbens*). The nylon bag is made of polyester measuring 8x4 cm with a porosity of 40µm. Feed samples were put into the rumen and incubated for 0, 4, 8, 12, 24, 48, and 72 hours. The parameters measured were consumption, patterns, and forage degradation characteristics by calculating the values of a, b, c, a+b, lag time, and ED. Determination characteristics of feed degradation in the rumen by *in sacco* method will be analyzed. The results showed that the characteristics of crude protein degradation had significant differences in fraction values a, b, a+b, and lag time ($P<0.05$), while c and ED did not have significant differences ($P>0.05$). In conclusion the crude protein of the degradation characteristics in the rumen were: elephant grass (a: 9.88%, b: 64.37%, and c: 0.06/h^{-1}), mini elephant grass (a: 16.50%, b: 45.24%, and c: 0.05/h^{-1}), guinea grass (a: 7.42%, b: 68.24%, and c: 0.05/h^{-1}), and signal grass (a: 6.79%, b: 56.19%, and c: 0.07/h^{-1}). So, grass can provide sufficient protein for microbial growth in the rumen for ruminants.

Keywords: Crude protein, Degradability, Forage, *in sacco*, Rumen.

INTRODUCTION

Forage is the main source of energy and basic needs for ruminants, in general term (Minson, 1990). Feed costs can reach 50% - 70% of the production cost of a ruminant livestock business (Bozic et al., 2012). The forage that is generally given to ruminants is derived from the grass or graminae group. Currently the main obstacle in increasing livestock production in tropical countries is the availability and quality of feed ingredients.

Ruminant farms in Indonesia generally use grass as the main feed which can be found nearby, for example elephant grass, mini elephant grass, guinea grass, and signal grass. The four grasses can be developed in tropical climates and have good nutrition to be used as feed for ruminants.

Feed is generally assessed for its quality, one of which is based on the protein content contained therein and aspects of degradation in the rumen. Provision of protein from forage to ruminants needs to pay attention to aspects of degradation in the rumen for microbial needs and those that escape microbial degradation (by-pass) as a source of protein to be utilized by the host (Puastuti et al., 2014). Protein degradation in the rumen is necessary to provide the N source needed for microbial growth (Mutsvangwa et al., 2016). Nichols et al. (2022) added that the contribution of microbial protein in the rumen plays an important role in sustaining N requirements in ruminants.

The *in sacco* method is a method for measuring the degradation value of a feed ingredient in the rumen (Mahrez and Ørksov, 1977; Guadayo et al., 2019). The degradation value of the sample will be measured with a nylon bag containing the sample and will be incubated in the rumen of fistula cattle at certain time intervals (Ørskov, 2000). It is important to know the quality of the protein content in elephant grass, mini elephant grass, guinea grass, and signal grass. It is necessary to conduct research to analyze the patterns and characteristics of feed degradation by using forage sources in livestock *in sacco*.

MATERIALS AND METHODS

Ethical approval

The experimental procedure for *in sacco* degradation of forage feed in live animals complies with the principles of animal welfare and was approved by the Health Research Ethics Committee, Hasanuddin University (Approved Number: 645 /UN4.6.4.5.31 /PP36 /2021, Protocol UH21090601) prior to this study held.

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Experimental animals and diets

This research was conducted from September 2021 to February 2022 at Hasanuddin University, Makassar, Indonesia. This study used the Latin Square Design method 4x4 which used 4 goats with fistulas of the male kacang variety (*Capra aegragus hircus*) aged ±2 years. The average weight of the goats used as test animals was 14.57±1.219 Kg. During the observation, the animals were given the same feed consisting of elephant grass, mini elephant grass, guinea grass, signal grass, and rice bran each of the goat's dry matter requirement, namely 3% of initial body weight (BB) given twice a day every morning and evening. Drinking water is provided *ad libitum.*

Experimental design

Observation of feed degradation characteristics was carried out using the *in sacco* method, using 8x4 cm nylon bags with 40µm porosity. The feed ingredients tested were R1: elephant grass (*Pennisetum purpureum*), R2: mini elephant grass (*Pennisetum purpureum cv. Mott*), R3: guinea grass (*Panicum maximum*), and R4: signal grass (*Bracharia decumbens*) which were harvested 40 days after the last pruning. The grass will be dried in an oven (60^{o}C) for 24 hours and ground to a size of ± 2 mm then tested for proximate analysis (AOAC, 1995) and analysis of fiber components (Van Soest, 1994) the results can be seen in table 1. Each sample of feed ingredient 3 grams was put in a nylon bag and then incubated in the rumen with an incubation period of 0, 4, 8, 12, 24, 48, and 72 hours. This study used the Latin Square Design 4×4 randomization method which consisted of four observation periods using four goats and four different forages. The incubated bag was put into the oven at 60°C for 48 hours. During this observation, each animal was given the same feed consisting of the 4 tested feed ingredients with the addition of 20% rice bran.

Calculation of crude protein content in feed before and after incubation using the Kjeldahl method (AOAC, 2001) at the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The loss of crude protein in samples from nylon bags during each incubation period is assumed to be a protein that has been successfully degraded in the rumen used to calculate the degradation of crude protein feed according to the type of grass and the length of the incubation period. Determination of Crude Protein (PK) which is degraded in the sample can be known by multiplying the percentage of PK content in the initial sample. The formula for calculating the percentage loss of sample PK is presented as follows: % PK Loss = (%Initial PK x Initial Sample Weight) – (%Final PK x Final Sample Weight)

Furthermore, the crude protein lost during the incubation period is used to measure the value of Y by calculating the values a, b, c and a+b which are entered into the exponential equation according to Ørskov and McDonald (1979) as follows:

$$Y = a + b\,(1-e^{-ct})$$

Where:

Y= Feed degradation by rumen microbes at time t (incubation time); a= soluble fraction; b= Potentially degraded fraction; c= Potential fraction degradation rate (b); a + b= Total potential degradation, including material that escapes the codend without degradation; t_0= Incubation time at 0 hours

The effectiveness of degradation is measured by the equation according to Orksov and McDonald (1979): $ED = a + [(b \times c)/(c + k)]$, assuming the feed flow rate (k) is 0.05/h (Srakaew et al., 2021). Effectively degraded crude protein is assumed to be Rumen Degradable Protein (RDP). Rumen Undegradable Protein (RUP) from each sample is calculated by the following equation: RUP = 100% − RDP (Terefe et al. 2022). Determination of the degradation curve and feed characteristics in the rumen *in sacco* will be analyzed using the Neway program (Ismartoyo, 2011). Data analysis uses One-Way Analysis of Variance (ANOVA), and if there are differences, then continue with Duncan's test. Statistical data used Software Package for Social Sciences (SPSS) Version 25. Then used Microsoft Excel 2010 software to see the degradation curve.

RESULTS AND DISCUSSION

Feed consumption

Consume fresh ingredients in livestock is at an average of 78.52 g/Kg BW/day. According to McDonald et al. (2002) factors that influence consumption in livestock are the characteristics of feed ingredients, environment, and livestock conditions. Han et al. (2019) added that palatability greatly affects the consumption of feed in livestock. The dry matter intake in this study was around 460.59 g/day or 61.68 g/Kg$^{0.75}$/day. These results are lower than those obtained by Mbuthia and Gachuiri (2003), who obtained a value of 79 g/Kg$^{0.75}$/day, but higher than the results of Manaye et al. (2009) which was only 57.9 g/Kg$^{0.75}$/day and Han et al. (2019) who obtained a value of 369.9 g/day. The normal range of dry-meter intake per kilogram of metabolic bodyweight in goats is in the range of 34–104 g/Kg (Decandia et al., 2007). The voluntary intake of crude protein in goat poo is 56.33 g/day, or 7.55 g/Kg^{0,75}/day. These findings are similar to those obtained by Sultana et al. (2012), whose crude protein intake for goats ranged from 45.2 to 75.7 g/day, and Baete and Aregheore (2011), whose crude protein intake for grass was 7 g/Kg^{0,75}/day.

Crude protein degradation

Table 1 shows that the incubation period of 4, 8 and 12 hours of the four grasses had no significant difference

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(P<0.05). Whereas at 24, 48, and 72 hours incubation showed significant differences (P<0.05) from the various feeds tested. In the duncan follow-up test, 24-hour incubation of elephant grass showed a high percentage of degradation compared to other feeds and at 48 and 72 hours of incubation, degradation of crude protein in elephant grass and guinea grass showed no different results but showed significantly different results from mini elephant grass and signal grass. The loss of crude protein in the nylon bag during a certain incubation period indicates that the 72 hour incubation period is the peak of feed degradation in the rumen for forage feed. These results are in accordance with those reported by Akhirany et al. (2013) that the peak of degradation of fibrous forages in the rumen was at an incubation period of 72 hours. Based on Figure 1 which shows the curve or pattern of crude protein degradation *in sacco* on four forages. From the curve it can be seen that there is an increase in the percentage of crude protein degradation as the incubation period increases. Increased degradation of crude protein in the rumen occurs during the incubation period of 4 to 24 hours. Meanwhile, during the 48 to 72 hour incubation period, the pattern of rumen degradation of crude protein began to stabilize. After 48 to 72 hours of incubation, the degradation pattern began to stabilize because the feed substrate had decreased in the rumen, which is similar to that obtained by Guadayo et al., (2019) and Jiang et al., (2020). This is in accordance with the opinion of Zulkarnain et al. (2014) an increase in the incubation period in the rumen reduces the feed substrate due to degradation by microbes.

Table 1 - Nutritional content of feed

Chemical composition	Elephant grass	Mini elephant grass	Guinea grass	Signal grass	Bran
Dry matter (%)	28.02	26.29	23.63	33.04	90.30
Organic material (%)	82.23	82.82	88.41	90.81	85.32
Crude protein (%)	14.79	12.13	11.19	15.31	7.71
Crude fiber (%)	31.84	27.44	33.42	31.02	32.19
NDF (%)	66.22	62.71	68.14	68.24	49.65
ADF (%)	41.23	36.90	42.24	39.70	38.80
Cellulose (%)	36.17	32.80	35.36	35.36	22.13
Hemicellulose (%)	24.99	25.81	25.90	28.54	10.85
Lignine (%)	2.08	2.05	3.30	2.66	8.85

Source: Feed Chemistry Laboratory Analysis Results, Faculty of Animal Husbandry, Hasanuddin University 2022.

Characteristics degradation of forage crude protein

The degradation characteristics consist of the fraction that is easily soluble in water or degraded (a), the fraction that is not soluble in water but can be degraded (b), and the rate of degradation of the fraction b (c), the total potential for degradation (a+b), and the lag time. Each feed ingredient has different characteristics from one another

The characteristics of crude protein degradation in table 2 show that the fraction a, or the easily soluble fraction in the four grasses tested gave significantly different results (P<0.05). The "a" fraction in mini elephant grass (16.5% ± 0.671) was the highest compared to the other materials studied. The “a” fraction relates to the solubility of plant cell contents in water (Zulkarnain et al., 2014). This result can be attributed to the lower ADF content of mini elephant grass compared to the other three grasses. It was also reported by Katongole et al. (2021) that the high fraction of crude protein (CP) which is easily degraded by the rumen in forage feed can be affected by the low ADF content in forage. The lignin content of the forage greatly reduces the degradability of the cell wall (Hatfield & Kalscheur, 2020) because lignin cannot be broken down by microbes in the rumen (Wang et al., 2022).

The crude protein fraction that was difficult to degrade or “b” in the four grasses tested showed a significant difference (P<0.05). The "b" fraction in Guinea grass was the highest (68.24%±2.301) when compared to the other grasses tested. these results indicate that the value of a and fraction b are negatively related, a similar thing was reported by Larbi et al. (1996) and Bamikole et al. (2004) showed that forages with higher a values had lower b values. This fraction can be related to the tannin content in plant cells which can form tannin protein complex bonds so as to reduce protein degradation in the rumen. Min et al. (2003) and Patra and Saxena (2010) suggested that tannins can reduce the degradation of the protein fraction of feed in the rumen.

The value of c is the rate of degradation of fraction b. the results of the analysis of variance showed that the c values for the four grasses tested were not significantly different (P>0.05). The rate of degradation of fraction b (c) on signal grass was an average of $0.07/h^{-1}$ which was the highest compared to other grasses. The average degradation rate of all tested grasses was $0.05/h^{-1}$ which is consistent with the assumed rate of feed fraction in the rumen (Ajayi et al., 2007). From the results obtained, it was found that the fractional degradation value and the degradation rate had a negative relationship, namely a low feed degradation rate resulted in a high degradation value. This was also reported by Odedire et al. (2013) and Rasjid and Ismartoyo (2014), and Ferreira et al. (2014).

Table 2 shows the lag time of the several tested feeds showing significantly different results (P<0.05). From Duncan's test it can be seen that mini elephant grass has the highest lag time compared to the other three grasses, namely 4.2 hours. The longest lag time is 4.2 hours, while the fastest lag time is 1.2. These results indicate the time required for microbes to adapt to the feed substrate in the rumen. However, the results of this lag time cannot be used as an index to see the degradation of a fibrous feed ingredient because in this case the value of the fraction of the feed will

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be negative (Ørksov et al., 1980; Odedire et al., 2013).

Table 2 - Consumption of livestock voluntary feed

Goat	Intake as feed basis (g/day)	DM Intake (g/day)	CP Intake (g/day)
Goat-1	1245.14±43.29	501.29±17.43	61,31±2,13
Goat-2	1154.67±93.54	464.87±37.66	56.85±4.61
Goat-3	1049.97±76.75	422.72±30.90	51.70±3.78
Goat-4	1126.33±41.40	453.46±16.67	55.46±2.04
Average	1144.03	460.59	56.33

Description: Feed consists of 20% elephant grass + 20% mini elephant grass + 20% guinea grass + 20% signal grass + 20% rice bran

Degradation effectiveness

The effectiveness of degradation (ED) is the result of the accumulation of feed degradation characteristics such as easily degradable fraction (a), slowly degraded fraction (b), and degradation rate of b fraction (c). EDCP on the four feeds showed results that were not significantly different (P>0.05). The effectiveness of crude protein degradation in the rumen of the four treatments showed a higher EDCP value at R1, namely 40.29% ± 2.47 although according to statistical tests it does not show the difference. This can be seen from the NH3 level which is a product of feed protein content that is degraded by proteolytic enzymes in the rumen (Sari et al., 2021). In study of Mushandri (2022), which measured the concentration of NH_3 from the same four types of feed, the NH_3 level in elephant grass was the highest, 4.64 mM compared to other grasses. The effectiveness of crude protein degradation in elephant grass obtained a value of 43.20% ± 2.47 which is different from the results obtained from previous studies, namely 34.53% (da Silva et al., 2021) and 53.9% (Katongole et al., 2021). Mini elephant grass has a degradation effectiveness value of 37.88% ± 4.81, which is different from the results obtained by other researchers, namely 46.81%/d (da Silva et al., 2021), and 64% (Orsoletta et al., 2017). For Guinea grass, the degradation effectiveness value was obtained as much as 40.33% ± 2.72, this is different from other studies which obtained results of 34.18% (Ogunwole et al., 2011) and 46.67% (Bonelli et al., 2013). Meanwhile, based on the calculation of the effectiveness of degradation, Signal Grass obtained a value of 39.32% ± 5.54, this value is higher than the results obtained by Lana et al. (2007) namely 16.4% and Terefe et al. (2022) namely 27.86%. These different results were obtained because differences in the nutrient content of the samples could be caused by lighting/climate (Ballare et al., 1997), leaf/stem ratio (Lemaire et al., 2020), processing with fermentation (Ferreira et al., 2014), the addition of other materials and differences in results can also be caused by differences in the methods used.

Table 3- The average percentage of CP degradation at each incubation period

Incubation Period (Hours)	Elephant Grass (%)	Mini Elephant Grass (%)	Guinea Grass (%)	Signal Grass (%)
0	6.80 ± 2.85	4.05±1.65	6.32±1.73	2.68±0.91
4	19.58±1.70	12.75±1.79	14.04±3.22	12.50±3.68
8	27.81±1.34	23.87±0.50	27.96±5.81	26.82±4.98
12	36.47±5.57	31.07±1.67	35.60±5.24	34.78±6.24
24	54.87±3.55^b	42.08±0.75^a	46.86±2.82^a	47.34±8.79^a
48	68.99±4.86^b	60.53±1.41^a	67.57±2.80^b	59.68±6.91^a
72	71.47±3.99^b	62.13±1.88^a	70.80±1.72^b	62.00±6.31^a

a,b,c,d: Means in the same row with different superscripts differ significantly (P<0.05)

Table 4 - Characteristics of crude protein degradation

Parameters \ Feeds	R1	R2	R3	R4
A (%)	9.88±0.38^b	16.50±0.67^a	7.42±1.09^c	6.79±0.35d
B(%)	64.37±4.85^{ab}	45.24±11.37^c	68.24±2.301^a	56.19±5.183^b
A+B (%)	74.25±4.85^a	61.74±11.37^b	75.66±2.31^a	62.98±5.17^b
C (/H)	0.06±0.012	0.05±0.006	0.05±0.008	0.07±0.016
Lt (h^{-1})	1.2±0.84^b	4.2±0.08^a	1.3±1.02^b	1.2±1.07^b
Ed (%)	43.20±2.47	37.88±4.51	40.33±2.72	39.32±5.54
Rup (%)	56.80±2.47	62.12±4.51	59.67±2.72	60.68±5.54

R1: elephant grass (*pennisetum purpureum*), R2: mini elephant grass (*pennisetum purpureum cv. Mott*), R3: guinea grass (*panicum maximum*), R4: signal grass (*brachiaria decumbens*), a: soluble fraction, b: potential degradation fraction, a+b: total potential degradation, c: degradation rate of fraction b, lt: lag time, ed: degradation effectiveness, rup: rumen undegradable protein. Different superscripts in the same row show significant differences (p<0.05)

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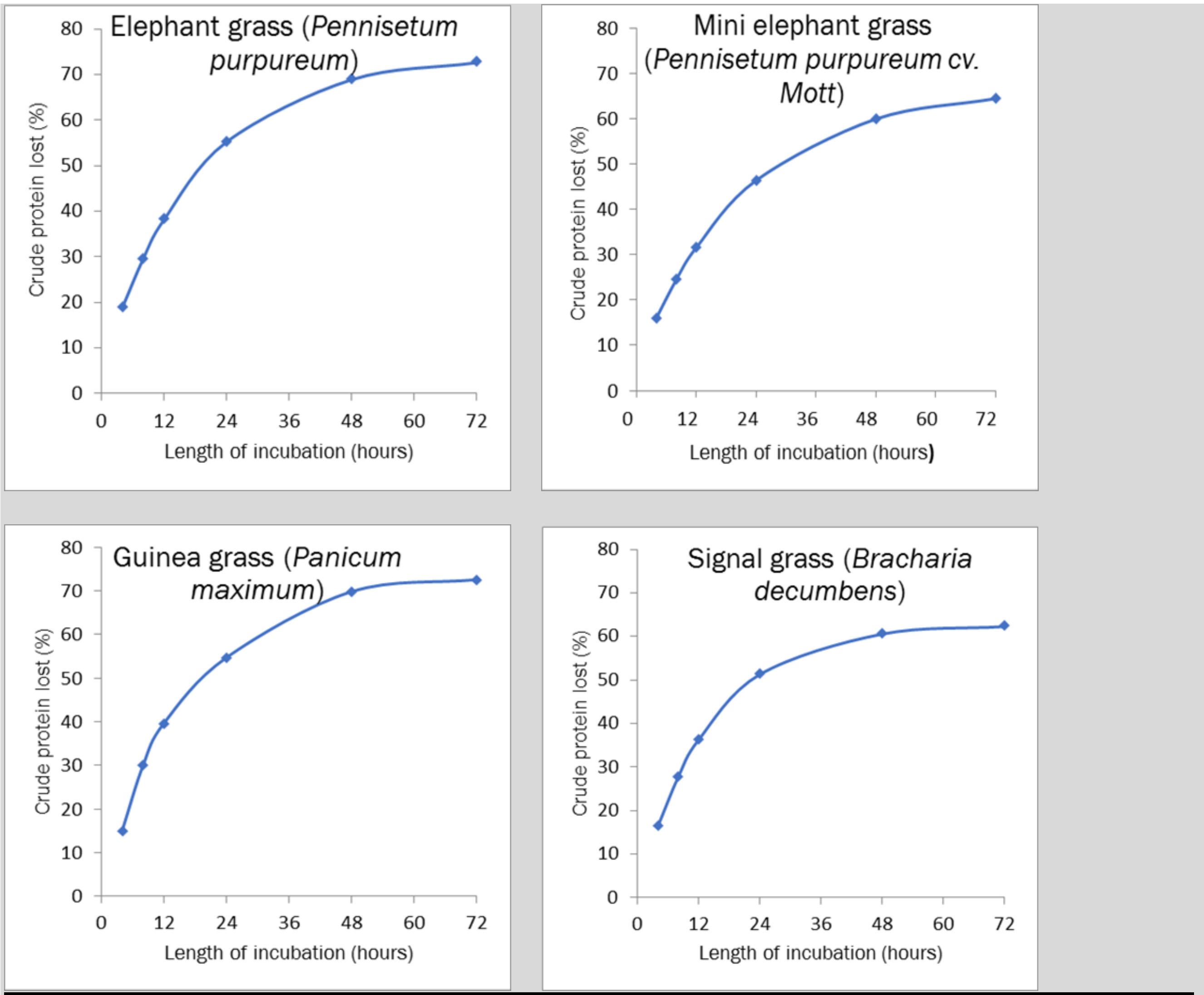

Figure 1 - Crude protein degradation curve Elephant grass (*Pennisetum purpureum*), Mini elephant grass (*Pennisetum purpureum cv. Mott*), Guinea grass (*Panicum maximum*), Signal grass (*Bracharia decumbens*)

CONCLUSION

In the results of this study, various grasses had suitable nutritional value and showed that the crude protein of the four grasses showed degradation characteristics: elephant grass (a: 9.88%, b: 64.37%, c: 0.06/h^{-1}), grass mini elephant (a: 16.50%, b: 45.24, and c: 0.05/h^{-1}), guinea grass (a: 7.42%, b: 68.24%, and c: 0.05/h^{-1}), and signal grass reached (a: 6.79%, b: 56.19%, and c: 0.07/h^{-1}). Of the four grasses, it was concluded that they were able to supply the protein requirements for microbial growth in the rumen and the protein needs of the livestock themselves.

DECLARATIONS

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Authors' contribution
Al Wijaya contribute Data collection, data analysis and the write up of the manuscript. I Ismartoyo and A. Natsir contribute on experiment, idea and research design.

Conflict of interests
The authors have not declared any conflict of interests.

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