Effect of rumen degradable protein and sulfur supplementation on in vitro digestibility and ruminal fermentation

A Rosmalia, Astiani, W P Sahroni, I G Permana*, Despal

Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia.

*Email: permana@apps.ipb.ac.id

Abstract. The availability of nitrogen and sulfur in the rumen should be synchronized to generate microbial protein synthesis. This study aimed to evaluate rumen degradable protein and sulfur supplementation on in vitro digestibility and ruminal fermentation. The experimental design was a 4 x 3 factorial randomized block design. Factor 1 was RDP levels (%CP) (R1= 60%; R2= 55%; R3= 50%; R4= 45%), and factor 2 was sulfur supplementation (S1= 0%; S2= 0.1%; S3= 0.2%). Data were analyzed with ANOVA followed by the Duncan test. The result showed that sulfur supplementation improved DMD and OMD without change in rumen pH. NH₃ concentration was influenced by RDP levels. Treatment R1 had the highest NH₃ concentration (7.03 mM). There was an interaction of two factors on total VFA concentration. The combination of R1 and S2 resulted in the highest total VFA concentration than others. The total bacteria population increased with increasing RDP levels. Protozoa population was affected by RDP levels and sulfur supplementation. This study concluded that a combination of 60% RDP and 0.2% sulfur supplementation resulted in the best ration to improve ruminal fermentation and digestibility.

1. Introduction
Feed is very important for dairy cattle development in Indonesia. The quality of dairy feedstuff is varied due to the tropical climate. In the ruminant, protein can be divided into rumen degradable protein (RDP) and rumen undegradable protein (RUP). RDP is degraded to ammonia as a source of nitrogen (N) which is used by rumen microbes to create their body (protein synthesis) [1]. The excess ammonia will be absorbed through the rumen wall then formed urea in the liver and excreted as urine [2]. The minimum requirement of RDP is 60% of total crude protein (CP) [3]. Some studies revealed that high RDP in dairy rations (more than 60% of CP) can improve milk production [4,5]. High RDP level (70% of CP) increased organic matter and crude protein digestibility [6]. Previous studies reported that low RDP levels can affect ruminal fermentation and reduce milk production [7,8].

Sulfur is known as an essential mineral in dairy nutrition that is needed for sulfur-containing amino acid synthesis, especially methionine, cysteine, cystine, homocysteine, cystathionine, taurine, and cysteic acid. It also plays a role in vitamin synthesis such as thiamine, biotin, and lipoic acid [3,9]. Rumen microbes require a sufficient amount of sulfur mineral for protein synthesis that should be available in the ration. According to NRC [3], the sulfur requirement is 0.14%-0.26% of dry matter (DM). Supplementation of sulfur ranged from 0.12% to 0.18% in the ration is needed to maintain sulfur
retention in mid-lactating dairy cows [10]. Sulfur supplementation at 0.16% of DM ration increased in vitro digestibility [11]. Sulfur supplementation can be carried out by adding synthetic sulfuric amino acids, sodium sulfate, calcium sulfate, ammonium sulfate, sulfuric acid, or elemental sulfur [12]. The addition of Na$_2$SO$_4$ and H$_2$SO$_4$ in the ration as a source of sulfur (S) can reduce methane emission [13]. Sulfur supplementation with Na$_2$SO$_4$ in the ration can improve ruminal fermentation and fiber digestibility [14].

The relationship between nitrogen and sulfur has been well documented to improve microbial protein synthesis and animal performance [12,15–18]. It has been reported that the optimal ratio of nitrogen and sulfur for microbial protein synthesis is 15:1 [15]. However, synchronization of RDP and sulfur supplementation in the dairy ration is not widely studied, especially in the tropical region. Moreover, the sulfur content in the most dairy ration is deficient due to the large proportion of agricultural by-products used. Therefore, sulfur supplementation is needed to overcome the problem. The objective of this study was to evaluate the effect of rumen degradable protein and sulfur supplementation on digestibility and ruminal fermentation using in vitro method.

2. Materials and methods

2.1. Location and experimental diet

This study was conducted from February to May 2021 at the Laboratory of Dairy Nutrition, Faculty of Animal Science, IPB University, Indonesia. The experimental diet was formulated through a combination of a 4 x 3 factorial design with the first factor was RDP levels that expressed as the percentage to CP (R1= 60%; R2= 55%; R3= 50%; R4= 45%) and the second factor was sulfur supplementation (S1= 0%; S2= 0.1%; S3= 0.2%). Na$_2$SO$_4$ was used as a source of sulfur (S) which contained 0.22% S. The diet contained 50:50 forage to concentrate ratio (DM basis). The nutrient contents of the diets were determined according to the AOAC method [19]. Feed composition and nutrient contents of the diets were presented in Table 1 and Table 2.

| Feed                  | R1 S1 | R1 S2 | R1 S3 | R2 S1 | R2 S2 | R2 S3 | R3 S1 | R3 S2 | R3 S3 | R4 S1 | R4 S2 | R4 S3 |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Napier grass          | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 |
| Corn                  | 2.00  | 2.00  | 2.00  | 2.00  | 2.00  | 2.00  | 4.19  | 5.13  | 6.07  | 13.74 | 15.12 | 16.49 |
| Rice bran             | 8.23  | 5.58  | 2.94  | 12.47 | 10.26 | 8.04  | 16.80 | 14.75 | 12.70 | 7.69  | 5.71  | 3.62  |
| Pollard               | 15.00 | 15.00 | 15.00 | 9.28  | 9.18  | 9.09  | 3.00  | 3.00  | 3.00  | 0.09  | 0.10  | 0.20  |
| Cassava meal          | 1.91  | 3.71  | 5.52  | 6.38  | 8.05  | 9.72  | 5.66  | 6.19  | 6.72  | 1.00  | 1.00  | 1.00  |
| SBM                   | 5.00  | 5.00  | 5.00  | 5.00  | 5.00  | 5.00  | 1.85  | 1.93  | 2.01  | 0.20  | 0.30  | 0.34  |
| CGM                   | 2.02  | 2.15  | 2.27  | 5.37  | 5.51  | 5.65  | 9.00  | 9.00  | 9.00  | 10.73 | 10.68 | 10.64 |
| Copra meal            | 10.00 | 10.00 | 10.00 | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 0.05  | 0.09  | 0.21  |
| Coffee husk           | 4.34  | 4.56  | 4.77  | 7.00  | 7.00  | 7.00  | 7.00  | 7.00  | 7.00  | 15.00 | 15.00 | 15.00 |
| CaCO$_3$              | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  |
| DCP                   | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  |
| Na$_2$SO$_4$          | 0.00  | 0.50  | 1.00  | 0.00  | 0.50  | 1.00  | 0.00  | 0.50  | 1.00  | 0.00  | 0.50  | 1.00  |

R1= 60% RDP; R2= 55% RDP; R3= 50% RDP; R4= 45% RDP; S1= 0% sulfur supplementation; S2= 0.1% sulfur supplementation; S3= 0.2% sulfur supplementation; SBM= soybean meal; CGM= corn gluten meal.
Table 2. Nutrient contents of the diets (%DM).

| Items   | S1  | S2  | S3  | S1  | S2  | S3  | S1  | S2  | S3  |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| DM      | 91.73| 92.13| 92.26| 92.05| 92.18| 91.72| 92.33| 91.98| 91.55| 92.10| 91.32| 92.71|
| ash     | 11.27| 11.36| 11.22| 11.81| 11.30| 11.86| 11.58| 11.60| 11.19| 10.14| 10.27| 10.02|
| EE      | 1.51 | 2.27| 2.17| 1.16| 0.99| 1.19| 1.26| 1.11| 1.25| 0.88| 1.04| 1.03|
| CP      | 12.73| 14.11| 13.79| 14.73| 13.58| 14.39| 13.97| 14.37| 13.56| 13.80| 15.10| 15.49|
| CF      | 24.31| 24.05| 22.76| 23.20| 23.55| 22.97| 23.03| 24.23| 23.64| 27.02| 24.50| 24.55|
| NFE     | 50.19| 48.21| 50.06| 49.11| 50.58| 49.58| 50.15| 48.69| 50.37| 48.17| 49.09| 48.91|
| TDN*    | 62.06| 63.25| 64.23| 62.92| 62.32| 63.10| 63.07| 61.95| 62.51| 59.38| 61.90| 61.94|

R1= 60% RDP; R2= 55% RDP; R3= 50% RDP; R4= 45% RDP; S1= without sulfur supplementation; S2= 0.1% sulfur supplementation; S3= 0.2% sulfur supplementation; DM= dry matter; EE= ether extract; CP= crude protein; CF= crude fiber; NFE= nitrogen free extract; TDN= total digestible nutrient; *TDN was calculated according to Sutardi equation using formula TDN=2.79+(1.17 x %CP)+(1.74 x %EE)-0.295 x %CF-0.81 x %NFE [20].

2.2. In vitro method and samples analysis

In vitro technique used two stages in vitro according to Tilley and Terry method [21]. The rumen fluid as a source of inoculant rumen microbes was taken from fistulated Frisian Holstein bulls before morning feeding. The amount of 0.5 g treatment diets samples was weighed and put into the fermentor tube. The amount of 40 mL McDougall buffer solution and 10 mL rumen fluid were added to the fermentor tube. The tube was aerated for 15 seconds with CO2 to make anaerobic conditions, then placed in a water shaker bath at 39 °C for 48 h. After 4 h incubation, the supernatant was collected for rumen pH and rumen microbes determination. After that, the fermenter tube was added with 2 drops of HgCl2 to stop the fermentation activity. The tube was centrifuged for 15 minutes at 3000 rpm. The supernatant was collected and stored chill for determination of NH3 and total VFA concentration. For digestibility measurement, the fermentation lasted for 48 h, and then the fermentation was stopped by added 2 drops of HgCl2. The tube was centrifuged for 15 minutes at 3000 rpm. The supernatant was removed and the residue was added with 50 mL 0.2% pepsin-HCl solution. The tube was incubated in a 39 °C water shaker bath for 48 h aerobically. After 48 h incubation, the residue was filtered using pre-determined weight Whatman paper no. 41 with assisted a vacuum pump. The residue was dried in a 105 °C oven for 24 h to determine dry matter residue and incinerated in a 650 °C oven furnace for 4 h to determine ash residue.

Dry matter and organic matter digestibility (DM and OM) were calculated by subtracting DM and OM residue from samples. Rumen pH was measured by a pH meter (Hanna, HI98191). The NH3 concentration was analysed using the micro diffusion Conway method and the total VFA was analysed using the steam distillation method [22]. Rumen bacteria and protozoa population were determined using Ogimoto and Imai method [23,24]. Rumen bacteria was measured after being cultured in BHI media. Before being cultured in the media, samples of rumen bacteria were diluted 4 times in glycerol media. Protozoa population was calculated using the colouring method and counted under a microscope with 4 x 10 magnification.

2.3. Statistical analysis

The data were analyzed using analysis of variance (ANOVA) continued by Duncan's multiple range test with SPSS software 20 version (IBM SPSS Statistics, USA).
3. Results and discussion

3.1. Ruminal fermentation characteristics

Ruminal fermentation characteristics can be described by rumen pH, ammonia (NH₃) concentration, volatile fatty acids (VFA) concentration, and rumen microbes population (bacteria and protozoa). Rumen pH is an indicator of rumen condition which affects the fermentation process and microbial activity [25]. NH₃ concentration is formed by degradation of protein or non-protein nitrogen (NPN). Total VFA is derived from organic matter fermentation especially carbohydrates [26]. The effect of RDP levels and sulfur supplementation on ruminal fermentation characteristics was presented in Table 3.

| Parameters | RDP levels | Sulfur supplementation | Average±SD |
|------------|------------|------------------------|------------|
| pH         |            |                        |            |
| R1         | S1         | 6.86±0.05              | 6.90±0.04  |
| R2         | S2         | 6.98±0.11              | 6.97±0.02  |
| R3         | S3         | 6.97±0.09              | 6.96±0.02  |
| R4         | Average±SD | 6.94±0.06              | 6.96±0.02  |

| NH₃ (mM)   |            |                        |            |
| R1         | S1         | 7.99±1.22              | 7.03±0.86  |
| R2         | S2         | 6.28±0.32              | 5.99±0.25  |
| R3         | S3         | 5.74±0.65              | 5.65±0.21  |
| R4         | Average±SD | 5.52±1.11              | 5.42±0.36  |

| Total VFA (mM) | R1         | 99.39±9.06 <sup>cd</sup> | 117.30±32.87 |
| R2         | 138.64±7.14 <sup>ab</sup> | 114.92±20.91 |
| R3         | 135.75±3.95 <sup>b</sup> | 116.71±26.23 |
| R4         | 87.65±0.75 <sup>d</sup> | 97.63±8.72 |
| Average±SD | 115.36±25.70 | 121.44±25.74 |

| Rumen bacteria (log CFU ml<sup>-1</sup>) | R1         | 10.94±0.42              | 10.75±0.20 <sup>*</sup> |
| R2         | 10.60±0.31              | 10.67±0.10 <sup>*</sup> |
| R3         | 10.38±0.53              | 10.29±0.10 <sup>b</sup> |
| R4         | 9.59±0.34               | 9.69±0.09 <sup>c</sup> |
| Average±SD | 10.38±0.57              | 10.34±0.46 |

| Protozoa (log cell ml<sup>-1</sup>) | R1         | 6.15±0.07               | 6.10±0.10 <sup>*</sup> |
| R2         | 6.06±0.10               | 6.05±0.04 <sup>a</sup> |
| R3         | 6.09±0.14               | 6.03±0.05 <sup>a</sup> |
| R4         | 5.92±0.08               | 5.88±0.06 <sup>b</sup> |
| Average±SD | 6.05±0.10 <sup>a</sup> | 5.95±0.09 <sup>b</sup> |

Different superscripts in the same row and column show significant differences (p<0.05). R1= 60% RDP; R2= 55% RDP; R3= 50% RDP; R4= 45% RDP; S1= without sulfur supplementation; S2= 0.1% sulfur supplementation; S3= 0.2% sulfur supplementation; VFA= volatile fatty acids.

Rumen pH was not significantly affected by RDP levels and sulfur supplementation (p>0.05). This is in line with other studies that reported rumen pH did not affect by different RDP levels [27,28] and sulfur supplementation [16,17]. The rumen pH value in all treatments was in the normal range (6.86-7.00) to support the fermentation process. The ideal rumen pH to maintain microbial activity is 6.0-7.0 [29]. Some factors that can change rumen pH including feed composition, feed processing, buffering capacity, and length of forage chop [30].
The NH₃ concentration was influenced by RDP levels \( (p<0.05) \). Sulfur supplementation did not affect NH₃ concentration \( (p>0.05) \). There was no interaction between RDP levels and sulfur supplementation on NH₃ concentration \( (p>0.05) \). Treatment R1 had the highest NH₃ concentration. Treatment R1 (60% RDP) supplied more RDP which increased nitrogen available, thus it increased NH₃ concentration in the rumen [31]. In the rumen, RDP is broken down by proteolytic microbes into peptides, amino acids, and NH₃. The NH₃ as a source of N is used by bacteria to synthesize microbial protein [32]. Davies et al. [33] reported that NH₃ concentration \( \geq 5.0 \) mg dl\(^{-1} \) or \( \geq 2.93 \) mM was the minimum required for microbial protein synthesis. The average NH₃ concentration in this study ranged from 5.02 mM-7.99 mM. This indicates protein could be degraded by rumen microbes and the amount of NH₃ was available for microbial growth.

The interaction between RDP levels and sulfur supplementation affected total VFA concentration \( (p<0.05) \). The combination between treatments R1 (60% RDP) and S2 (0.1% sulfur supplementation) had the highest total VFA concentration. According to Putri et al. [28], increasing total VFA concentration was followed by increasing RDP in the rumen. Total VFA concentration was increased by sulfur supplementation with Na₂SO₄ due to attributed increasing cellulolytic bacteria population [14]. Total VFA consists of acetate, propionate, butyrate, iso-butyrate, valerate, and iso-valerate. A high cellulolytic bacteria population tends to increase acetate production [34]. Total VFA is influenced by some factors including substrate availability, rate of rumen fermentation, feed consumption, VFA, absorption, and liquid or solid passage [35].

The dairy ration with different RDP levels affected the rumen bacteria population \( (p<0.05) \), but sulfur supplementation did not affect the rumen bacteria population \( (p>0.05) \). Treatment R1 and R2 (60% and 50% RDP) had a higher rumen bacteria population than other treatments. A high RDP level provides more N which is needed for microbial growth [36]. The high bacterial population found in this study is in line with high NH₃ concentration as an impact of high RDP levels. A previous study revealed that high RDP levels increased microbial N production which is related to the high rumen bacteria population [37]. The forage to concentrate ratio has been reported to have influenced the rumen bacteria population [38]. The rumen bacteria population in this study was in the normal population range according to McDonald et al. [26] which is 9-10 log CFU ml\(^{-1} \).

Protozoa population was affected by RDP levels and sulfur supplementation \( (p<0.05) \), but there was no interaction between the two factors \( (p>0.05) \). Protozoa population was the lowest in R4 treatment due to the low bacterial population. Protozoa act as predators for rumen bacteria, so the decreasing population of bacteria reduces the protozoa population [39]. Sulfur supplementation at 0.1% and 0.2% levels reduced protozoa population. Wu et al. [13] reported that sulfur supplementation decreased the methanogenic archaea population. Methanogens have a symbiotic association with protozoa by transferring hydrogen, thus decreasing methanogens reduced protozoa population [40]. Supplementation of sulfur in the dairy ration can be suggested to decrease methane emission by converting H₂ for methanogenesis to H₂S [18,41]. The protozoa population in this study ranged from 5.88-6.17 log CFU ml\(^{-1} \). The protozoa population was in the normal range population according to McDonald et al. [26] which is 5 – 6 log cell ml\(^{-1} \).

3.2. Dry matter and organic matter digestibility

Dry matter digestibility and organic matter digestibility (DMD and OMD) describe carbohydrate, protein, fat, and mineral that can be digested by rumen microbes and enzymes of post-ruminal. The DMD and OMD produced in this study were shown in Table 4.

There was no interaction between RDP levels and sulfur supplementation on DMD and OMD. The DMD and OMD were not affected by RDP levels \( (p>0.05) \), but it was affected by sulfur supplementation \( (p<0.05) \). Sulfur supplementation 0.1%-0.2% increased DMD and OMD. Eliharidas et al. [11] reported 0.16% sulfur supplementation increased nutrient digestibility (dry matter, organic matter, crude protein, and fiber) due to the increasing rumen microbe population. The addition of sulfur has been reported to stimulate the growth of cellulolytic bacteria [42]. In this study, DMD and OMD value was lower than reported by Lestari et al. [43] which reported DMD and OMD in the range 66.46%-70.71% and 69.13%-
75.47%, respectively, in the same forage to concentrate ratio. This might be due to the low crude protein content in the ration (±14.14%). Zahera et al. [44] reported that there is a positive correlation between crude protein and DMD or OMD.

**Table 4. Dry matter and organic matter digestibility.**

| Parameters | RDP levels | Sulfur supplementation | Average±SD |
|------------|------------|-------------------------|------------|
| DMD (%)    | R1         | 53.51±4.17              | 53.11±1.01 |
|            | R2         | 51.36±2.37              | 67.93±1.02 |
|            | R3         | 49.13±1.32              | 69.88±1.94 |
|            | R4         | 51.27±0.29              | 52.30±1.05 |
| OMD (%)    | R1         | 51.48                   | 51.36±1.27 |
|            | R2         | 49.88                   | 51.00±1.02 |
|            | R3         | 47.49                   | 50.03±2.21 |
|            | R4         | 48.73                   | 50.10±1.32 |

Average±SD 51.32±1.79b 52.60±0.58ab 53.00±0.90a

Different superscripts in the same row show significant differences (p<0.05). R1 = 60% RDP; R2 = 55% RDP; R3 = 50% RDP; R4 = 45% RDP; S1 = without sulfur supplementation; S2 = 0.1% sulfur supplementation; S3 = 0.2% sulfur supplementation; DMD = dry matter digestibility; OMD = organic matter digestibility.

4. Conclusions

The tropical dairy ration with a high RDP level (60%) and sulfur supplementation up to 0.2% improve in vitro digestibility and fermentability.

**Acknowledgement**

We sincerely acknowledge and thank the Indonesian Ministry of Research and Technology-National Research and Innovation Agency with PMDSU research scheme, contract No 077/SP2H/LT/DPRM/2021 and subcontract No 2881/IT3.L1/PN/2021 for financial support.

**References**

[1] Hristov A N, Bannink A, Crompton L A, Huhtanen P, Kreuzer M, McGee M, Nozière P, Reynolds C K, Bayat A R, Yáñez-Ruiz D R, Dijkstra J, Kebreab E, Schwarm A, Shingfield K J and Yu Z 2019 *J. Dairy Sci.* 102 5811–52

[2] Carvalho I P C d., Doelman J and Martín-Tereso J 2020 *J. Anim. Physiol. Anim. Nutr. (Berl).* 104 64–75

[3] NRC 2001 *Nutrient Requirements of Dairy Cattle* (Washington DC: National Academy Press)

[4] Savari M, Khorvash M, Amanlou H, Ghorbani G R, Ghasemi E and Mirzaei M 2018 *J. Dairy Sci.* 101 1111–22

[5] Kalscheur K F, Vi R L B, Glenn B P and Kohn R A 2006 *J. Dairy Sci.* 89 249–59

[6] Valizadeh A, Kazemi-Bonchenari M, Khodaei-Motlagh M, Moradi M H and Salem A Z M 2021 *Small Rumin. Res.* 197 106330

[7] Gressley T F and Armentano L E 2007 *J. Dairy Sci.* 90 1340–53

[8] Zanton G I, Heinrichs A J and Jones C M 2013 *J. Dairy Sci.* 96 4638–42

[9] Miller W J 1979 *Dairy Cattle Feeding And Nutrition* ed T J Cunha (London (UK): Academic Press)

[10] Bouchard R and Conrad H R 1973 *J. Dairy Sci.* 56 1276–82

[11] Elihasridades, Jamarun N, Zain M and Marlida Y 2012 *J. Peternak. Indones. (Indonesian J. Anim. Sci.* 14 349–54
[12] da Silva C J, Leonel F de P, Pereira J C, Costa M G, Moreira L M, de Oliveira T S and de Abreu C L 2014 Rev. Bras. Zootec. 43 537–43
[13] Wu H, Meng Q and Yu Z 2015 Bioresour. Technol. 186 25–33
[14] Xie B, Gao J and Zhao G 2020 Appl. Environ. Microbiol. 86 1–18
[15] Bal M A and Ozturk D 2006 Res. J. Anim. Vet. Sci. 1 33–36
[16] Supapong C, Cherdhong A, Wanapat M, Chanjula P and Uriyapongson S 2019 Animals 9 1–11
[17] Promkot C and Wanapat M 2009 Asian-Australasian J. Anim. Sci. 22 1366–76
[18] Rebelo L R, Luna I C, Messana T A, Granja-Salcedo Y T, Vito E S, Lee C, Teixeira I A M A, Rooke J A and Berchielli T T 2019 Anim. Feed Sci. Technol. 257 114293
[19] AOAC 2005 Official Methods of Analysis ed W Horwitz and G W Latime (Maryland (USA): AOAC International)
[20] Indah A S, Permana I G and Despal D 2020 Sains Peternak. 18 38
[21] Tilley J M A and Terry R A 1963 Grass Forage Sci. 104–11
[22] Riestanti L U, Retnani Y and Despal D 2020 IOP Conf. Ser. Earth Environ. Sci. 41 012037
[23] Hu W L, Liu J X, Ye J A, Wu Y M and Guo Y Q 2005 Anim. Feed Sci. Technol. 120 333–9
[24] Wahyudi A, Cahyanto M N, Soejono M and Bachruddin Z 2010 J. Indones. Trop. Anim. Agric. 35 34–41
[25] Pitt R E, Van Kessel J S, Fox D G, Pell A N, Barry M C and Van Soest P J 1996 J. Anim. Sci. 74 226–44
[26] McDonald P, Edwards R A, Greenhalgh J F D, Morgan C A, Sinclair L A and Wilkinson R G 2010 Animal nutrition (London (UK): Pearson)
[27] Pilachai R, Schonewille J T, Thamrongyoswittayakul C, Aiumlamai S, Wachirapakorn C, Everts H and Hendriks W H 2012 J. Anim. Physiol. Anim. Nutr. (Berl). 96 206–13
[28] Putri E M, Zain M, Warly L and Hermon H 2021 Vet. World 14 640–8
[29] Erdman R A 1988 J. Dairy Sci. 71 3246–66
[30] McCAughern J H, Mackenzie A M and Sinclair L A 2020 J. Dairy Sci. 103 9024–36
[31] Broderick G A and Reynal S M 2009 J. Dairy Sci. 92 2822–34
[32] Gulinski P, Salamończyk E and Młynek K 2016 Anim. Sci. Pap. Reports 34 5–24
[33] Davies K L, McKinnon J J and Mutsvangwa T 2013 Can. J. Anim. Sci. 93 123–36
[34] Zhao X Hui, Lui C Juann, Li C Yun and YAO J Hu 2013 J. Integr. Agric. 12 1471–80
[35] Hall M B, Nennich T D, Doane P H and Brink G E 2015 J. Dairy Sci. 98 3988–99
[36] Bach A, Calsamiglia S and Stern M D 2005 J. Dairy Sci. 88 E9–21
[37] Zhao X H, Gong J M, Zhou S, Fu C B, Liu C J, Xu L J, Pan K and Qu M R 2015 Ital. J. Anim. Sci. 14 220–5
[38] Wanapat M, Gunun P, Anantasook N and Kang S 2014 J. Agric. Sci. 152 675–85
[39] Dijkstra J, France J and Tamminga S 1998 J. Agric. Sci. 130 81–94
[40] Mebrate G, Tewodros A and Dawit A 2019 Concepts Dairy Vet. Sci. 2 2637–4749
[41] Richter E L 2011 The effect of dietary sulfur on performance, mineral status, rumen hydrogen sulfide, and rumen microbial populations in yearling beef steers (Iowa State University)
[42] McSweeney C S and Denman S E 2007 J. Appl. Microbiol. 103 1757–65
[43] Lestari D A, Abdullah L and Despal 2015 Media Peternak. 38 110–7
[44] Zahera R, Anggraeni D, Rahman Z A and Evvyernie D 2020 J. Ilmu Nutr. dan Teknol. Pakan 18 1–6