Effects of *Megasphaera elsdenii* supplementation on fermentation and lactic acid concentration in the rumen: A meta-analysis of in vivo experiments

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**Abstract.** This study evaluated the effects of supplementation *M. elsdenii* on rumen fermentation and lactic acid concentration *in vivo* by integrating data from various related studies using a meta-analysis. A database was developed by integrating data from 72 treatments that originated from 17 articles. The parameters integrated were lactic acid concentration, fermentation products, and rumen microbial population. The database compiled was statistically analyzed using a mixed model methodology. Different studies were considered as random effects, and the doses of *M. elsdenii* were treated as fixed effects. The significance of an effect was stated when its p-value was <0.05. Results showed that supplementation of *M. elsdenii* linearly reduced lactic acid concentration (P=0.048), proportion of acetate (P=0.045), acetate: propionate ratio (P=0.043) and methane production (P<0.01). In addition, *M. elsdenii* supplementation also had a significant quadratic effect to increase total VFA (P<0.01) and linearly with pH (P<0.01), proportion of propionate (P=0.037), and valerate proportion (P=0.037). However, supplementation of *M. elsdenii* did not significantly affect (P>0.05) isobutyrate, isovalerate proportion, and protozoa population in the rumen. It can be concluded that *M. elsdenii* supplementation is proven to reduce lactic acid concentration, maintain rumen pH, reduce methane production and increase some rumen fermentation products.

1. Introduction

Provision of quality feed with adequate nutrient supply is essential in livestock production [1–3], including the balance between forage to concentrate ratio. Switching abruptly from forage feed to excess concentrate feed can decrease rumen pH with symptoms of metabolic disorders such as acidosis. Acidosis is very important to prevent because it affects feed intake, rumen fermentation products, rumen microflora, milk production and can increase the risk of diarrhea, laminitis, and liver abscess [4]. Acidosis can be prevented in several ways, such as feed management by adapting feed, buffers, antibiotics, and giving probiotics. Probiotic supplementation is considered a strategic step in preventing acidosis by improving metabolism and the ability of rumen microbes to utilize lactate. The use of probiotics provides several other advantages, including improving the growth rate of livestock and improving feed use. This is achieved by increasing the efficiency of the digestive process. Bacteria as
probiotics can be classified as lactic acid producing bacteria, lactic acid user bacteria, and bacteria with other functions [5].

*Megasphaera elsdenii* is a lactate-consuming species that predominate in the rumen. *M. elsdenii* can ferment the L and D isomers of lactate through the racemase enzyme and is a major contributor in the control or prevention of lactic acidosis by utilizing lactic acid [6]. *M. elsdenii* reduced lactic acid concentrations in the rumen and increased feed intake, thereby improving daily body weight gain [7,8]. The decrease in lactic acid concentration can reach 20% on in vitro experiments [9] and 10% on in vivo experiments [8]. Therefore, it is necessary to study further the use of *M. elsdenii* to reduce lactic acid to prevent acidosis and its effect on fermentability in livestock. Based on the research that has been done, it is found that there are inconsistencies in the results obtained. However, these results can be mediated by using a meta-analysis method. This study aimed to evaluate the effectiveness of using *M. elsdenii* in vivo on the fermentability and concentration of lactic acid in the rumen to prevent acidosis in ruminants by integrating data from various experiments and analyzing the data by a meta-analysis method.

2. Materials and methods
A database was constructed from literatures and published research articles that reported the supplementation *M. elsdenii* on ruminants. The search engines of journal collections such as Scopus, Web of Science, PubMed, Google Scholar, and Science Direct in 2009–2021. The keywords used in the literature search were "Megasphaera elsdenii" and "rumen". Based on these keywords, 56 articles were found, then after evaluating the title, abstract and method on in vivo studies. A total of 17 articles were included [7,8,10–24] used to build the database. The selected published in vivo studies that reported the effects of supplementation of *M. elsdenii* on ruminal lactic acid production, volatile fatty acid (VFA) concentration, and microbial population were compiled in a database. The *M. elsdenii* were used from any strain on the ruminants. The *M. elsdenii* concentrations expressed as log 10 CFU.

Measurement of methane gas production using the methane production estimation formula with calculations using the partial VFA production profile [25]. The details of descriptive statistics of the database in the meta-analysis are summarized in table 1. The collected data were statistically analyzed using a mixed-model meta-analysis approach [26]. The analysis employed the PROC MIXED procedure of SAS 9.4 software. The studies were taken as the random effects, while the concentrations of *M. elsdenii* supplantations were taken as the fixed effects. The model statistics used were p-values, coefficient of determination, and the Akaike Information Criterion [27]. These models were considered significant at P<0.05.

**Table 1.** Descriptive statistics of the database used to evaluate the effect of supplementation *M. elsdenii* on rumen fermentability and lactic acid concentration in ruminants

| Response parameters | n | Min | Max | Mean | SD |
|---------------------|---|-----|-----|------|----|
| Level *M. elsdenii* (log10 CFU) | 42 | 8.65 | 12.64 | 11.14 | 1.19 |
| pH | 70 | 5.00 | 6.62 | 6.00 | 0.33 |
| Total VFA (mM) | 54 | 69.30 | 253.00 | 124.40 | 38.07 |
| C2 (%) | 58 | 22.50 | 78.90 | 55.29 | 11.21 |
| C3 (%) | 63 | 3.90 | 42.30 | 22.73 | 6.99 |
| C4 (%) | 58 | 2.00 | 40.40 | 14.49 | 6.93 |
| IsoC4 (%) | 22 | 0.24 | 2.65 | 1.28 | 0.65 |
| C5 (%) | 26 | 0.58 | 4.61 | 2.50 | 0.84 |
| IsoC5 (%) | 18 | 0.61 | 3.85 | 2.19 | 0.94 |
| Ratio C2:C3 | 58 | 1.16 | 5.77 | 2.81 | 0.99 |
| CH4 (mM) | 58 | 12.43 | 34.40 | 24.68 | 4.36 |
| Lactic acid (mmol/L) | 47 | 0.20 | 2.50 | 0.79 | 0.51 |
| protozoa (log 10) | 10 | 5.00 | 6.26 | 5.85 | 0.39 |

n: number of observations, SD: standard deviation, Min: minimum, Max: maximum, VFA: *Volatile Fatty Acid*, C2: acetate, C3: propionate, C4: butyrate; IsoC4: isobutyrate, C5: valerate, IsoC5: isovalerate, CH4: Methane gas
3. Results and discussion

The results descriptive statistics of database obtained were 72 treatments from 17 articles, that reported the use of *M. elsdenii* on rumen fermentability with various levels of administration between 8.65–12.64 (log 10 CFU) with an average administration of 11.14 ± 1.19 (log 10 CFU). All the response parameter results are presented in table 2. Based on the analysis results, it was found that the supplementation of *M. elsdenii* probiotics had a linear (P<0.01; R=0.41) significant effect on increasing pH. In addition, based on descriptive statistical analysis, it was found that the average rumen pH in this study was 6.00 ± 0.33. These results prove that the supplementation of *M. elsdenii* probiotics is able to maintain a pH ≥ 6. These results are consistent with several studies which reported that the administration of *M. elsdenii* was able to maintain rumen pH ≥ 6 after feeding grains [8,28]. Moreover, *M. elsdenii* strain NCIMB 41125 successfully prevented the decrease in rumen pH and accumulation of lactic acid in the rumen [9].

**Table 2. Regression equations of ruminal fermentation parameters on the supplementation of *M. elsdenii* in the in vivo ruminants’ experiments**

| Response parameters | n | M | Parameter estimates | Model estimates | IT |
|---------------------|---|---|----------------------|-----------------|----|
|                     |   |   | Int       | SE Int | Slope   | SE Slope | P value | R² | AIC |   |
| pH                  | 70 | L | 5.84      | 0.06   | 0.02    | 0.007    | <0.01   | 0.41| 40.7| + |
| Total VFA (mM)      | 54 | Q | 125.67    | 7.53   | 1.66    | 0.512    | <0.01   | 0.47| 341.1| + |
| C₂ (%)              | 58 | L | 58.62     | 2.16   | -0.53   | 0.256    | 0.045   | 0.65| 249.7| - |
| C₃ (%)              | 63 | L | 20.59     | 1.31   | 0.32    | 0.153    | 0.037   | 0.44| 259.2| + |
| C₄ (%)              | 58 | L | 12.64     | 1.33   | 0.34    | 0.157    | 0.033   | 0.49| 230.3| + |
| IsoC₄ (%)           | 22 | L | 1.20      | 0.21   | 0.02    | 0.024    | ns      | 0.38| 271.8| + |
| C₅ (%)              | 26 | L | 2.14      | 0.22   | 0.06    | 0.027    | 0.037   | 0.56| 44.5 | + |
| IsoC₅ (%)           | 18 | L | 2.05      | 0.34   | 0.02    | 0.036    | ns      | 0.57| 33.7 | + |
| Ratio C₂:C₃         | 58 | L | 3.11      | 0.19   | -0.05   | 0.02     | 0.043   | 0.46| 103.3| - |
| CH₄ (mM)            | 58 | L | 25.79     | 0.85   | -0.18   | 0.10     | <0.01   | 0.65| 187.1| - |
| Lactic acid (mmol/L)| 47 | L | 0.97      | 0.11   | -0.03   | 0.013    | 0.048   | 0.34| 56.3 | - |
| Protozoa (log 10)   | 10 | L | 5.74      | 0.2    | 0.01    | 0.02     | ns      | 0.17| 118.8| + |

n: number of observations, M: model, Int: Intercept L: linear, Q: quadratic, R²: coefficient of determination, AIC: akaike information criterion, IT: interpretation trend. VFA: Volatile Fatty Acid, C₂: acetate, C₃: propionate. C₄: butyrate; IsoC₄: isobutyrate, C₅: valerate, IsoC₅: isovalerate, CH₄: Methane gas

The analysis results showed that the use of *M. elsdenii* based on the analysis supplementation had a significant quadratic effect on the increase in total VFA (P<0.01; R²=0.47). The significant increase in total VFA was due to the high carbohydrate feed given to each treatment. These results proved that giving *M. elsdenii* was able to increase some of the rumen fermentation products. Moreover, the use of *M. elsdenii* had a linear effect of a significant reduction in the proportion of acetate (P=0.045; R²=0.65). The decrease in the proportion of acetate due to *M. elsdenii* at low rumen pH resulted in a higher proportion of propionate and butyrate than acetate. In addition, decreased the proportion of acetate because the acetate produced was re-fermented through the β-oxidation pathway to produce butyrate [16], so that the proportion of butyrate experienced a significant increase (P=0.033; R²=0.49) with the provision of this *M. elsdenii* probiotic. Based on the analysis results, it was also found that the proportion of propionate had a significant increase (P=0.037; R²=0.44) to the increase in the level of *M. elsdenii* probiotic. This is following some literature which states that giving *M. elsdenii* can increase propionate [7,10,29]. The proportion of acetate that decreased significantly and propionate which increased significantly caused the acetate propionate ratio (C₂:C₃) to decrease significantly (P=0.048; R=0.46). In addition, the production of CH₄ was obtained based on the estimation calculation [25] in table 2, where *M. elsdenii* supplementation significantly reduced methane gas estimation (P<0.01; R²=0.65). This result is because the formation of CH₄ and propionate both require H₂, so that an increase in propionate production will reduce methane production.

Excess carbohydrate intake during the fermentation process of transition from forage to concentrate feed causes lactic acid concentration to increase in the rumen. This disorder is mainly characterized by
the accumulation of organic acids resulting from fermentation in the rumen. The organisms that produce lactic acid are more than organisms that utilize lactic acid. It is estimated that *M. elsdenii* accounts for 60-80% of lactic acid metabolism in the rumen [30], making it an ideal candidate to help mitigate lactic acidosis. Therefore, *M. elsdenii* is an important species to control the occurrence of rumen acidosis. Based on the results of the analysis, it was found that the level of *M. elsdenii* probiotic supplementation had a significant reduction effect (P =0.048; R²=0.34) linearly on the concentration of lactic acid. This shows that the higher the level of *M. elsdenii* probiotic supplementation can reduce lactic acid concentration in the rumen. *In vivo* research conducted by McDaniel et al. [23] also found that cannulated steers with high concentrations of feed grain and inoculated with *M. elsdenii* had higher rumen pH and lower lactate concentrations than steers not given *M. elsdenii*. In addition, the analysis showed that *M. elsdenii* probiotic supplementation had no significant effect (P>0.05) on the protozoa population in the rumen. The role of protozoa in the rumen is as a stabilizing factor for fermentation, by ingesting bacteria to prevent fermentation, especially when given high grain feed [28].

4. Conclusions

The effect of *M. elsdenii* supplementation *in vivo* of various research data has been shown to be able to reduce the concentration of lactic acid, methane gas, and the proportion of acetate as well as to increase several rumen products such as total VFA, the proportion of propionate, butyrate, isobutyrate, and valerate significantly. Administration of *M. elsdenii* was able to maintain rumen pH after feeding grains.

Acknowledgment

This research was funded by Kementerian Riset dan Teknologi-Badan Riset dan Inovasi through World Class Research (WCR) grant, year 2021, contract number 077/SP2H/LT/DRPM/2021.

References

[1] Kondo M, Hirano Y, Kita K, Jayanegara A and Yokota H O 2014 *Asian-Australas J Anim Sci.* 27 937–45
[2] Kondo M, Shimizu K, Jayanegara A, Mishima T, Matsui H, Karita S, Goto M and Fujihara T 2016 *J Sci Food Agric.* 96 1175–80
[3] Suharlin, Astuti D A, Nahrowi, Jayanegara A and Abdullah L 2016 *Int. J. Dairy Sci.* 11 100–5
[4] Plazier J C, Krause D O, Gozho G N and McBride B W 2008 *Vet J.* 176 21–31
[5] Elghandour M M, Rasmus L J, Henning P H and Coertze R J 2010 *Australas. J Anim. Sci.* 50 49
[6] Henning P H, Horn C H, Leeuw K J, Meissner H H and Hagg F M 2010a *Anim. Feed Sci. Technol.* 157 20–9
[7] Meissner H H, Henning P H, Leeuw K J, Hagg F M and Horn C H 2014 *Livestock Science* 162 115–25
[10] Aikman P C, Henning P H, Humphries D J and Horn C H 2011 *J. Dairy Sci.* 94 2840–49
[11] Alatas M S and Umucalilar H D 2015 *International Journal of Animal and Veterinary Sciences* 9 776–81
[12] Hagg F M, Rasmus L J, Henning P H and Coertze R J 2010 *South African Journal of Animal Science* 40 101–12
[13] Long M, Li P, Zhang Y, Chen X, Gao Z and Lin G 2016 *Indian J. Anim. Res.* 50 330–4
[14] Sedighi R and Alipour D 2019 *Anim. Feed Sci. Technol.* 248 126–31
[15] Muya M C, Nherera F V, Miller K A, Aperce C C, Moshidi P M and Erasmus L J 2015 *J. Anim. Physiol Anim Nutr.* 99 913–8
[16] Weimer P J, Cabral L D S and Catite F 2015 *J. Dairy Sci.* 98 8078–92
[17] Zebeli Q, Terrill S J, Mazzolari A, Dunn S M, Yang W Z and Ametaj B N 2011 *J. Dairy Res.* 79
16–25

[18] Mazon G, Campler M R, Holcomb C, Bewley J M and Costa J H C 2020 *Anim. Feed Sci. Technol.* 261 114404

[19] Arik H D, Gulsen N, Armagan H and Alatas M S 2018 *J. Anim Physiol Anim Nutr.* 103 416–46

[20] Direkvandi E, Mohammadabadi T and Salem A Z M 2020 *Journal of Applied Animal Research* 48 235–43

[21] Ellerman T J, Horton L M, Katulski S L, Van Bibber-Krueger C L, Aperce C C and Drouillard J S 2017 *Journal of Animal Science* 95 277–8

[22] Thieszen J, Van Bibber C L, Axman J E and Drouillard J S 2015 *Kansas Agricultural Experiment Station Research Reports* 1 1–5

[23] McDaniel M R, Heidenreich J M, Higgins J J and Drouillard J S 2009 *Kansas Agricultural Experiment Station Research Reports* 1 62–5

[24] Klieve A V, McLennan S T and Ouwerkerk D 2012 *Animal Production Science* 52 297–304

[25] Moss A R, Jouany J P and Newbold J 2000 *Ann. Zootech.* 49 231–53

[26] St-Pierre N R 2001 *J. Dairy Sci.* 84 741–55

[27] Jayanegara A, Wina E and Takahashi J 2014 *Asian-Australas J Anim Sci* 27 1426–35

[28] Wiryawan K G and Brooker J D 1995 *Aust. J. Agric. Res.* 46 1555–68

[29] Prabhu R, Altman E and Eitemana M A 2012 *Appl Environ Microbiol.* 78 8564–70

[30] Counotte G H and Prins R A 1981 *Vet. Res. Commun.* 5 101–15