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RESEARCH ARTICLE



Environmental efficiency of *Saccharomyces cerevisiae* on methane production in dairy and beef cattle via a meta-analysis

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Abstract

The objective of the present study is to examine the effect of yeast (*Saccharomyces cerevisiae*) on reduction of methane (CH₄) production in dairy and beef cattle using meta-analytic methods. After compilation of relevant scientific publications available from the literature between 1990 and 2016, and applying exclusion and inclusion criteria, meta-analyses of data from dairy and beef cattle were applied for the pooled dataset or for each animal category (dairy or beef). The results of meta-analysis of all three datasets (all cattle, dairy cattle, or beef cattle) suggested that effect size of yeast either on daily CH₄ production or on CH₄ production per dry matter intake (CH₄/DMI) was not significant. The results of Q test and I^2 statistic suggest that there is no heterogeneity between different studies on CH₄ production and CH₄/DMI. The results of meta-analysis suggest that use of yeast (*Saccharomyces cerevisiae*) as feed additive does not offer significant results in terms of reduction of CH₄ production in dairy and beef cattle. Further research on the effects of different doses of yeast, use of yeast products, different strains, and experimental designs is warranted to elucidate the effects of yeasts on methane production in the rumen.

Keywords Yeast · Meta-analysis · Methane · Dairy cow · Beef cattle

Introduction

Undoubtedly, one of the significant sources of greenhouse gas emissions is livestock. Among the greenhouse gases, methane (CH₄) is one of the major gases produced out of enteric fermentation in ruminants and it has 25 times more potential of

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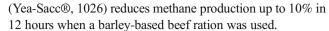
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global warming than carbon dioxide (CO₂; Kataria 2015). Beside of adverse implications of CH₄ production on energy efficiency of ruminants, the gas has raised some concerns of environmental contamination in past few years. About 2 to 12% of gross energy of the feed could be lost through CH₄ (Johnson and Johnson 1995). The reticulo-rumen is a fermentation chamber containing a complex and diverse microbiota composed of different microbial communities. Such as bacteria, archaea, protozoa, and fungi. All of them involved in the microbial fermentation of feed. Methane production occurs due to the metabolic activity of methanogenic archaea capable of utilizing autotrophically hydrogen and CO₂ as substrates (Bayat et al. 2015; Meller 2016). Therefore, in past few years numerous studies have been conducted concerning possible reduction of CH₄ production per unit of meat and milk yielded by ruminants. This has led to the development of numerous strategies aiming to reduce CH₄ production by ruminants. In terms of ruminant nutrition, some solutions such as modification of concentrate, type and quality of forage, defaunation, and use of ionophores, oils, organic acids, and direct fed microbial and prebiotics have been proposed (Boadi et al. 2004; Iqbal et al. 2008).



One of the alternatives for reduction of CH₄ production by ruminants that has drawn a considerable attention in recent years is the use of yeasts, as one type of direct fed microbes or probiotics. Yeast products have been used as feed additives for ruminants to improve production performance (increase of growth rate, meat, and milk) and to alleviate acidosis thus improving animal health and welfare (Chaucheyras-Durand et al. 2012; Vohra et al. 2016). As a natural feed additive, yeasts contribute to balance and stabilize rumen microbiota, to maintain a favorable pH and enhance the formation of fermentation end-products in the rumen, and to improve ammonia utilization by ruminal bacteria (Chaucheyras-Durand et al. 2012). Previous meta-analyses investigating the effects of yeast additives on ruminants (Desnoyers et al. 2009), in particular dairy cattle (Poppy et al. 2012), suggest that the use of yeast as supplement could increase milk production. Regarding the effects of yeast supplements on production performance of beef cattle, the results of the meta-analysis reported by Sartori et al. (2017) suggested that adding yeast to the rations fed to beef cattle could reduce dry matter intake (DMI) but has no significant effects on average daily gain. This effect might be dependent on dosage of yeasts, strain of yeasts, and diet composition. The previously conducted studies point to insignificant effects of yeast products on reduction of CH₄ production of dairy and beef cattle. The review of results on the association between CH₄ production and CH₄ emission intensity (CH₄/DMI) shows some controversial outcomes. Regarding dairy cattle, Muñoz et al. (2016) and Chung et al. (2011) added active dried yeast, Meller (2016) added live yeast culture, and Bayat et al. (2015) added live yeast to the feed but found no significant change in CH₄ production. However, the studies conducted by Bayat et al. (2015) and Chung et al. (2011) suggested that the use of yeast decreased CH₄ production, although the difference from unsupplemented diets was insignificant. One of the supplementation strains used in the feed of beef cattle was suggested by McGinn et al. (2004) as reducing CH₄ production but the difference was not significant. Regarding the results of CH₄ emission intensity, Muñoz et al. (2016) reported that addition of yeast was followed by higher yield of CH₄/DMI and digestible organic matter intake. In contrast, Chung et al. (2011) found out that one strain of Saccharomyces cerevisiae tended to cause a relative reduction of CH₄ production when compared with other strains.

The results of *in vitro* assays on the effect of yeast on reduction of methane production are contradictory compared with results from *in vivo* studies. Hernández et al. (2017) suggested that 2 and 4 mg yeast/g DM feed could reduce methane production by dairy calves if they are fed concentrate rations. In another study, it was reported that a mixture of yeast and a high-dose mixture of xylanase offers the best results in terms of reduction of methane produced by calves (Hernández et al. 2017). Mutsvangwa et al. (1992) suggested that a yeast culture



In general, the use of yeast products as an additive to dairy and beef cattle rations could improve performance. However, CH₄ mitigation by yeast could be a relatively ambiguous and contradictory matter (Hristov et al. 2013). Although *in vitro* experiments are also regarded as a valuable way of testing ideas, *in vivo* experiments could offer more realistic results. Therefore, in this meta-analysis, only data from *in vivo* reports were reviewed and used. Since meta-analysis could summarize holistically results of different studies (Sutton and Higgins 2008), the objective of the present paper is to survey the influence of yeast (*Saccharomyces cerevisiae*) on CH₄ production as well as CH₄/DMI in dairy and beef cattle through meta-analytical methods.

Material and methods

Literature search

An extensive, structured and systematic literature search was carried out using databases of ISI Web of Knowledge (http:// wokinfo.com) and Google Scholar (http://scholar.google. com). The publication period of studies was from January 1990 to December 2016. The keywords used for search of relevant studies included "(dairy cow OR beef) AND methane AND (yeast OR Saccharomyces cerevisiae)." In order to assure the compilation of all relevant studies on the topic, references of collected papers were reviewed (Lean et al. 2009). In the case that experimental results were reported in dissertations, these were also included in the reviewed literature. More than 100 scientific publications were identified and all of them were initially screened for acceptability by determining if the research conducted studied effects of yeast (Saccharomyces cerevisiae) on CH₄ production. After discarding papers that were not directly related with the topic, an initial data set of 46 publications was compiled.

Inclusion and exclusion criteria

After collection of studies published between 1990 and 2016, the reports in dairy and beef cattle were further screened for a subsequent selection. Such studies should include both a yeast-recipient (treated) group and a control unsupplemented group (no yeast administered). In addition, only studies with detailed *in vivo* measurement of CH₄ production were included. The reviews (n = 10) as well as *in vivo* experiments on influence of yeast on production parameters and CH₄ production of other animals (sheep and goat; n = 15) and *in vitro* experiments (n = 14) were excluded from the database.



Data extraction

Data were screened and extracted independently by two investigators to identify and determine if a publication was to be included in the meta-analysis. Data used in the present meta-analysis were those for mean CH₄ production or mean CH₄/DMI. In addition, other data used were SE (standard error), and number of cows in treatment and control groups. Many studies reported a common SE and these estimates were used for both control and treatment groups. Other information such as name of author, year of publication, CH₄ measurement method, and breed of cows, type of yeast products, and nutritional ration was also recorded. Quality assessment was conducted before data extraction in accordance with the recommendations of Lean et al. (2009).

Statistical analysis

Effect size and forest plots

Comprehensive Meta-Analysis Software (version 2.2, Biostat, USA) was used for the statistical analyses. The effect size for daily CH₄ production and CH₄/DMI for all included studies (dairy and beef cattle, dairy cattle, and beef cattle) was determined as standardized mean difference (SMD) at 95% level of confidence intervals. One of the methods used for continuous data are SMD estimation (Lean et al. 2009). The SMD is the mean difference between treatment and control groups which is standardized based on standard deviation (SD) of treatment and control groups and result is a numerical dimensionless value. The SMD enable comparison of differences between groups regarding several variables and the model adopted in this meta-analysis was a random effects model (Borenstein et al. 2009). Random effects models have an underlying assumption that a distribution of effects exists, resulting in heterogeneity among study results (Borenstein et al. 2009). If SD was not reported in studies, it was calculated by multiplying the standard error of means by the square root of the number of cows.

Forest plot is one of the common plots used in metaanalysis which represents information of each study as well as final outcome of all the studies (Lean et al. 2009). Points to the left of the vertical line represent a reduction in the outcome, and points to the right of the line indicate an increase in the outcome variable. Each square represents the mean effect size for that study. The upper and lower limit of the line connected to the square represents the upper and lower 95% confidence intervals for the size effect. In this meta-analysis, CH₄ production and CH₄/DMI of all studies (dairy and beef cattle) were developed into a forest plot. In forest plot, effect size is equal with SMD at 95% confidence interval in the case of adopting random model.

Assessment of heterogeneity

In order to test heterogeneity across studies, $\chi^2(Q)$ test and I^2 statistic were used (Borenstein et al. 2009). Variations among the study level were assessed using a Q test (formula: 1).

Formula 1 :
$$Q = \sum_{i=1}^{k} w_i (Y_i - M)^2$$
,

where w_i is the study weight, Y_i is the study effect size, M is the summary effect, and k is the number of studies (Borenstein et al. 2009). Since power of Q test in meta-analytical studies with low number of studies is insignificant, level of significance was presumed to be equal to 0.1 (Lean et al. 2009). Although Q test contributes to the detection of heterogeneity, the quantitative value (percentile form) is determined through I^2 statistic (Higgins and Thompson 2002). The I^2 is a transformation of the square root of the Q heterogeneity divided by its degrees of freedom and describes the proportion of total variation in study estimates that is due to heterogeneity. Negative values of I^2 are equalized to zero, consequently I^2 lies between 0 and 100%. If I^2 exceeds 50 percent, the parameters will be presumed to have significant heterogeneity (Lean et al. 2009).

Publication bias

Publication bias was examined through funnel plot and trim and fill method (Duval and Tweedie 2000). The plot is concerned with estimated index of each study compared with its precision sample size. Therefore, larger size of the study is correlated with its higher precision; such studies are represented at the top part of the plot. The studies with smaller size are represented in the lower part of the plot (Lean et al. 2009). In the case that Funnel plot showed bias from one or more of the studies used, number of studies to be excluded and adjusted effect size were determined through trim and fill method. In this meta-analysis, CH₄ production and CH₄/DMI of all studies (dairy and beef cattle) were developed into a funnel plot.

Results

Characteristics of the database

Table 1 identified the publications selected and the data extracted for the meta-analysis. In general, the database included 6 studies out of which 4 studies were related to dairy cattle and the remaining 2 studies were associated with beef cattle. Although number of comparisons between yeast-recipient group and control group made up of beef cattle is low (i.e., 3 comparisons), a meta-analysis with a minimum of three comparisons is possible (Valentine et al. 2010). One should note that the results lack high statistical power and results should be interpreted with caution. In some experiments on dairy cattle (Chung et al. 2011;



 Table 1
 Summary of publications used for meta-analysis

Reference	NC ¹	Animal	Breed	Yeast products	CH ₄ measurement method	Feed	Response variables
Bayat et al. 2015	2	Dairy cow	Finnish Ayrshire	Live yeast	Sulfur hexafluoride	TMR ²	CH ₄ , CH ₄ /DMI
Chung et al. 2011	2	Dairy cow	Holstein	Active dried yeast	Sulfur hexafluoride	TMR	CH ₄ , CH ₄ /DMI
Hristov et al. 2010	1	Dairy cow	Holstein	fermented yeast culture	Sulfur hexafluoride	TMR	CH ₄
Meller 2016	1	Dairy cow	Jersey	Live yeast culture	Chamber	TMR	CH ₄ , CH ₄ /DMI
Muñoz et al. 2016	2	Dairy cow	Holstein-Friesian, Norwegian and Norwegian ×Holstein- Friesian	Active dried yeast	Chamber	TMR	CH ₄ , CH ₄ /DMI
McGinn et al. 2004	2	Beef cattle	Holstein	Active dried yeast	Chamber	TMR	CH ₄ , CH ₄ /DMI
Possenti et al. 2008	1	Beef cattle	Crossbred	Active dried yeast	Chamber	roughage	CH ₄ , CH ₄ /DMI

In the plot (Fig. 1), the average difference between yeast-receiving group and control group for each study is represented by a square and confidence intervals 95% is shown by a transverse line

Bayat et al. 2015) or beef cattle (Possenti et al. 2008), yeast was placed in rumen of animals through cannula tubes. Other studies added yeast to the feed. In the database, Bayat et al. (2015), Chung et al. (2011), and Hristov et al. (2010) measured CH₄ using the sulfur hexafluoride (SF6) method. Other studies measured CH₄ emission using respiratory chambers. Among the experiments with dairy cattle, Chung et al. (2011) conducted experiments on non-lactating cows, whereas in the other experiments, the cows were in lactation. Among the experiments on beef cattle, McGinn et al. (2004) conducted a test on growing beef cattle and Possenti et al. (2008) used cross-bred cows. In all of the experiments included in present meta-analysis, the yeast used was *Saccharomyces cerevisiae*.

CH₄ production

The results of the meta-analysis of the effect of yeast on CH_4 production by dairy and beef cattle are shown in Table 2. The

meta-analytic results of 11 comparisons between yeast-recipient group and control group suggested that the effect of yeast addition on reduction of CH₄ production did not reach statistical significance when dairy and beef cattle were pooled (SMD = -0.051; P = 0.792). The difference between yeast and control groups were not significant for dairy cows (SMD = -0.083; P = 0.708) or for beef cattle (SMD = 0.057; P = 0.889). As shown in Table 2, the Q test and P-statistic showed that there was no heterogeneity across studies on CH₄ production. The P value in the Q test for CH₄ production in dairy and beef cattle, dairy cattle, and beef cattle was greater than 0.1 and P was zero.

The forest plot of CH₄ production (Fig. 1) showed a final outcome in terms of SMD.

The results of publication bias obtained through review of funnel plot (Fig. 2) and trim and fill method suggest that there is no publication bias in terms of CH₄ production by dairy and beef cattle. In this plot, effect index of small studies will lower precision is represented at the bottom of the plot.

Table 2 Effect size and heterogeneity for effect of yeast on CH₄ production and CH₄/DMI in dairy cows and beef cattle

Outcome	NC^1	SMD^2	95% confidence intervals	P value	Q	P value	I^2
CH ₄ production				,			
Both dairy cows and beef cattle	11	- 0.051	- 0.435, 0.331	0.792	5.488	0.856	0
Dairy cows	8	- 0.083	- 0.517, 0.351	0.708	4.773	0.688	0
Beef cattle	3	0.057	- 0.748, 0.863	0.889	0.625	0.732	0
CH ₄ /DMI							
Both dairy cows and beef cattle	10	-0.087	- 0.566, 0.392	0.722	10.550	0.308	14.689
Dairy cows	7	- 0.120	- 0.807, 0.566	0.732	10.042	0.123	40.252
Beef cattle	3	0.002	- 0.802, 0.806	0.996	0.437	0.804	0

In the plot (Fig. 1), the average difference between yeast-receiving group and control group for each study is represented by a square and confidence intervals 95% is shown by a transverse line

² SMD standardized mean difference



¹ NC number of comparisons

² TMR total mixed rations

¹ NC number of comparisons

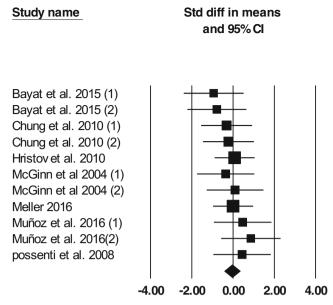


Fig. 1 Forest plots of the effect sizes (std diff in means and 95% CI: standardized mean differences and 95% confidence intervals) between yeast (*Saccharomyces cerevisiae*) supplementation versus no supplementation for CH₄ production in dairy cows and beef cattle. The mean effect size, calculated according to a random effects model, is indicated by the diamond at the bottom. The size of the squares illustrated the weight of each study relatively to the mean effect size. Smaller squares represent less weight

CH₄/DMI

The results of the meta-analysis of the effect of yeast in dairy and beef cattle on CH_4/DMI are shown in Table 2. The results

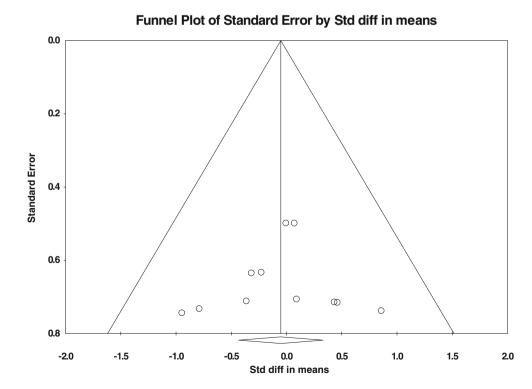
suggest that the effect of yeast on reduction of CH₄/DMI did not reach statistical significance when dairy and beef cattle data were pooled (SMD = -0.087; P = 0.722), with only dairy cattle data (SMD = -0.120; P = 0.732), or with only beef cattle data (SMD = 0.002; P = 0.996). No significant heterogeneity was found across studies on CH₄/DMI. The P value in the Q test was greater than 0.1. Although the value of I^2 for dairy cattle was about 40%, the I^2 was zero for beef cattle. The forest plot (Fig. 3) of CH₄/DMI shows the final outcome as SMD.

The results of publication bias (CH₄/DMI, Fig. 4) of dairy and beef cattle suggest that there is one study missing. Trim and Fill method suggested that one possible additional study was required to generate a normal response distribution (adjusted effect size= 0.004; 95% confidence intervals= -0.467, 0.477). Similar to previous plot (Fig. 2), the studies are clustered at the bottom of the plot.

Discussion

Meta-analysis is defined as application of different statistical methods to summarize results from several studies through combination and statistical analysis of pooled data. Different review and meta-analytic studies examining the effects of using yeast on production performance of dairy and beef cattle have been reported previously. For instance, the meta-analytical study of Desnoyers et al. (2009) suggested that supplementation of yeast causes an increase of ruminal pH level

Fig. 2 Funnel plots of the effect sizes (std diff in means: standardized mean differences) for CH₄ production. Empty circles indicate observed values





Study name

Std diff in means and 95% CI

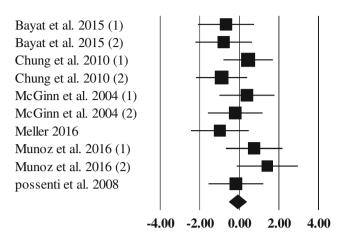


Fig. 3 Forest plots of the effect sizes (std diff in means and 95% CI: standardized mean differences and 95% confidence intervals) between yeast (*Saccharomyces cerevisiae*) supplementation versus no supplementation for CH₄/DMI in dairy cows and beef cattle. The mean effect size, calculated according to a random effects model, is indicated by the diamond at the bottom. The size of the squares illustrated the weight of each study relatively to the mean effect size. Smaller squares represent less weight

and concentration of volatile fatty acids in rumen but reduces concentration of lactic acid in rumen and has no effects on acetate to propionate ratio in ruminants. In addition, supplementation of yeast increases DMI, milk production, and milk

fat without influencing milk protein content. In a metaanalysis of the effects of yeast culture on dairy cattle, Poppy et al. (2012) suggested that supplementation of yeast culture product increases milk production, increase of 3.5% fat corrected milk, and contributes to milk fat yield as well as milk protein yield. Sartori et al. (2017) conducted a metaanalysis of effect of supplementation of live yeast on performance of beef cattle and reported that addition of yeast did not affect average daily gain but DMI was reduced. Therefore, despite of positive effect of yeasts as reported in previous meta-analytic studies, there are few in vivo experiments on the effect of yeast (Saccharomyces cerevisiae) on CH₄ production. The results of present meta-analysis suggest that adding yeast to ration of dairy and beef cattle did not reduce CH₄ production. The experiments of Bayat et al. (2015) and Chung et al. (2011) on dairy cattle suggested that CH₄ production (g/day) of yeast-receiving cows is less than in control cows although these differences were not significant. McGinn et al. (2004) conducted a similar experiment on beef cattle and found out that only one yeast-receiving group had produced lower CH₄ than the control group. In other experiments, no reduction of CH₄ production in yeast-receiving groups was found when they were compared with the control group. The result of each test could be represented in a forest plot (Fig. 1). In the forest plot, total outcome is shown by a diamond shape at the bottom of the plot.

In regard to CH_4/DMI , the results of the present metaanalysis suggest that supplementation of yeast did not reduce of CH_4/DMI . This result is consistent with the finding of other studies used in this meta-analysis. The forest plot (Fig. 3)

Fig. 4 Funnel plots of the effect sizes (std diff in means: standardized mean differences) for CH₄/DMI

Funnel Plot of Standard Error by Std diff in means 0.0 0.2 Standard Error 0.4 0 00 00 0.8 -2.0 -1.5 -1.0 0.0 0.5 1.0 1.5 2.0 Std diff in means



shows the result of each experiments as well as total outcome as SMD so that the total outcome is -0.087; P = 0.722.

Although supplementation of yeast did not reduce CH₄ production significantly, the result of *in vivo* experiments in sheep suggested that *Trichosporon sericeum* yeast culture reduced daily CH₄ production to a greater extent than control group (33.4 vs. 37.2 l/day). However, no significant reduction of CH₄/DMI was observed (Mwenya et al. 2004). In the case of growing goats, the results suggest that a combination of cellulase and *Saccharomyces cerevisiae* fermentation products reduces CH₄/DMI (Lu et al. 2016). It seems that in sheep and goats the influence of yeast on reduction of CH₄ production could be more positive. However, further experiments on sheep and goat is required to confirm this inference.

Another important parameter related to methane production in the rumen is the acetate to propionate ratio. Moss et al. (2000) suggested that while acetate and butyrate formation stimulate methane production, propionate could act as an alternative pathway for hydrogen sink in the rumen. Mutsvangwa et al. (1992) suggested that reduced production of methane with yeast-containing rations might be due to a greater propionate production requiring the use of metabolic hydrogen and therefore, it reduces methanogenesis. In this regard, Shibata and Terada (2010) suggested that the use of probiotics for ruminants changes the molar proportions of volatile fatty acids so that the proportion of acetate is decreased whereas that of propionate increases. Saccharomyces cerevisiae yeast might stimulate acetogenic bacteria which could use metabolic hydrogen in the rumen. This event deviates hydrogen from methanogenesis consequently (Chaucheyras-Durand et al. 2010). Chung et al. (2011) is the only study suggesting that the cows receiving yeast in their rumen show a significant reduction of the acetate-propionate ratio.

The present meta-analysis did not show heterogeneity with daily CH₄ production. In the case of CH₄/DMI, the I^2 for dairy and beef cattle was about 14% and for dairy cattle only the index was 40%. Such level of heterogeneity is not considered significant (Lean et al. 2009). The differences among experiments on the effect of yeast on CH₄ production and CH₄/DMI might be due to diverse factors such as strain of yeast, viability, feed intake, and/or management (Chaucheyras-Durand et al. 2012). The comparative study with two yeast strains (Levucell SC and a novel strain) versus a control group did not report a significant difference between strains in CH₄ production (Chung et al. 2011). Bayat et al. (2015), Chung et al. (2011), and McGinn et al. (2004) used two strains of yeast. Their findings suggested that CH₄ production (g/day) and amount of CH₄/DMI did not show a significant difference between supplemented and control cows. Regarding the type of yeast product, two studies used yeast culture. The cows receiving yeast culture did not show a significant decline in CH₄ production or CH₄/DMI in comparison with the control group (Meller 2016). Elghandour et al. (2017) conducted an *in vitro* experiment and suggested that differences between yeast cultures in terms of methane production might be due to their differing contents of protein, fat, fiber, and other materials.

In addition to abovementioned cases, the type of experimental design might also affect the results. In the database used in our meta-analysis, the experiment conducted by Chung et al. (2011) was based on randomized block design but the rest of the experiments were based on a cross-over design. Such design may have limitations for examining the effect of yeast on rumen-related parameters since yeasts could be influential upon equilibrium of microbial population (Bayat et al. 2015). In addition, Bayat et al. (2015) conducted a study on the effect of two strains of yeast on CH₄ production in dairy cattle and suggested that a slight reduction of CH₄ production occurred in yeast-receiving treatments. The decrease was not significant and this might have been due to the small number of observations.

The technique used in measurement of produced CH₄ could be another factor contributing to difference in reported results of different studies. In the present meta-analysis, studies used either SF6 or respiratory chamber techniques. Management factors, climate and physiological stage of the animal might be also influential upon results. As a consequence, one may conclude that although addition of yeast could exert a positive influence on production performance and ruminal parameters of cows, results of *in vitro* studies could not offer a valid strategy for investigating the reduction of methane

Conclusion

The results of present meta-analysis of three groups (all (pooled) dairy and beef cattle, dairy cattle only, or beef cattle only) showed that supplementation of yeast does not significantly reduce CH₄ production or CH₄/DMI. In addition, no significant heterogeneity was observed between different studies. These results should be interpreted with caution because they were based on a small number of studies. It is recommended that future experiments be conducted on other yeast products, different strains of yeasts, and different doses of yeast so as that *in-vivo* effect of yeast on livestock could be elucidated.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.



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