UNIVERSIDAD AUTÓNOMA DEL ESTADO DE MÉXICO
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INFLUENCE OF SACCHAROMYCES CEREVISIAE ON THE ENVIRONMENTAL EFFICIENCY OF METHANE PRODUCTION IN DAIRY AND BEEF CATTLE: META-ANALYSIS

"INFLUENCIA DE SACCHAROMYCES CEREVISIAE EN LA EFICIENCIA AMBIENTAL DE LA PRODUCCIÓN DE METANO EN GANADO LECHERO Y GANADO DE ENGORDA": META-ANÁLISIS

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PRESENTA
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DEDICATORIAS

A mis padres esposa hijos y hermanos por todo el apoyo comprensión paciencia y consejos dados armas de la ayuda que me brindaron durante los momento difíciles y todos los sacrificios hechos para ayudarme a culminar este largo camino A donde quiera que te encuentres este logro también es tuyo papá.

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CONTENIDO

1. INTRODUCCIÓN

El uso de aditivos alimentarios (p. ej., productos de levadura) para la reducción de la producción de metano (CH_4) de los rumiantes ha llamado la atención de los nutricionistas y ecologistas del ganado. Aunque hay muchos estudios relevantes sobre este tema, los resultados obtenidos para el ganado lechero y de carne son contradictorios. Por lo tanto, el objetivo del presente estudio es examinar el efecto de la levadura (*Saccharomyces cerevisiae*) en la reducción de la producción de CH_4 en ganado lechero y ganado de carne utilizando métodos metaanalíticos. Después de la compilación de artículos relevantes publicados entre 1990 y 2016 y la aplicación de criterios de exclusión e inclusión, se aplicaron meta análisis de datos de ganado lechero y ganado de engorda para el conjunto de datos agrupado o para cada categoría animal (lácteos o vacunos). El tamaño del efecto de la levadura sobre la producción de CH_4 (g / día) y la producción de CH_4 por ingestión de materia seca (CH_4 / DMI (g / kg)) se estimó como diferencia de medias estandarizada con un intervalo de confianza del 95%. Además, la heterogeneidad entre los estudios se evaluó mediante el uso de la prueba Q y el índice I^2. El sesgo de estudio se verificó a través de un gráfico Funnel y un método de ajuste y relleno. Los resultados del meta análisis de los 3 grupos de animales (todos los bovinos, vacas lecheras y bovinos) sugirieron que el tamaño del efecto de la levadura en la producción de CH_4 (g / día) y CH_4 / DMI (g / kg) no fue significativo. En otras palabras, el uso de levadura para ganado lechero y ganado de engorda no redujo significativamente la producción de CH_4 y CH_4 / DMI (g / kg). Los resultados de la prueba Q y el índice I^2

sugieren que no hay heterogeneidad entre diferentes estudios sobre producción de CH_4 y CH_4 / DMI (g / kg), por lo que el valor del índice I^2 de CH_4 / DMI (g / kg) en ganado lechero y ganado de carne alrededor del 14%, mientras que para el ganado lechero, el índice es de alrededor del 40%. No hubo sesgo de estudio de la producción de CH_4 ni de CH_4 / DMI (g / kg). Como se muestra en la gráfica de embudo, los estudios están más concentrados en la parte más baja de la gráfica. Los resultados del meta análisis sugieren que el uso de levadura (*Saccharomyces cerevisiae*) como aditivo en la alimentación no ofrece resultados significativos en términos de reducción de la producción de CH_4 en ganado lechero y ganado de carne. Se recomienda realizar más estudios sobre el efecto de diferentes dosis de levadura, el uso de productos de levadura, diferentes cepas y diseños experimentales.

2. REVISIÓN BIBLIOGRÁFICA

2.1 Levaduras

La levadura es un microorganismo que pertenece a la familia de los hongos y es utilizado por la humanidad desde hace tiempo. Es importante aclarar que si bien todas se llaman levaduras existen miles de cepas diferentes las cuales son específicas para cada labor (panificación, destilería, producción de extractos de levadura y uso en animales). De igual manera cuando hablamos de productos para uso animal existen muchos y muy diferentes entre sí por su composición y precio, razón por la cual el productor debe hacer una elección basada en algunos factores como son: Funcionan, características propias y calidad del producto, seriedad de la empresa productora, disponibilidad de investigación seria y reconocida donde se demuestre la funcionalidad de lo que le ofrece (Alvarado 2011).

Fuller (1989); los define como probióticos, microorganismos vivos que incluidos en la alimentación de los animales afecta positivamente al organismo receptor, mejorando su sistema digestivo. Las levaduras vivas de uso zootécnico son posiblemente el grupo de microorganismos que más se ha estudiado y que mayor auge ha tenido durante las dos últimas décadas en la producción bovina.

Las levaduras, son microorganismos pertenecientes al grupo que corresponden los probióticos. (Dawson 1993), otra definición dice que los probióticos son aditivos no nutritivos, los cuales contienen diferentes preparaciones de levaduras (muertas, de panificación y los cultivos de levaduras) con efectos diversos sobre la actividad ruminal, tasa de digestibilidad de los componentes de la dieta, porcentaje de degradabilidad del forraje, cambios en el patrón de fermentación ruminal, modificación del pH ruminal, cambios en el número de microorganismos del rumen e interacción bacterias (Arambel y Kent, 1990).

Las levaduras aportan enzimas esenciales, vitaminas y aminoácidos durante la digestión, algunos beneficios pueden surgir de los metabolitos per sé o por su interacción con otros organismos de la flora ruminal, mejorando el aprovechamiento de las fuentes nitrogenadas, tales como amonio y proteínas por parte de los microorganismos ruminales (Wholt et al., 1998),y los cuales tiene un efecto positivo sobre la respuesta positiva de los rumiantes (producción de leche y/o carne) (Wallace y Newbold, 1993).

2.2 PARTICULARIDADES DE LA DIGESTIÓN RUMINAL

La particularidad de los rumiantes radica en que son capaces de alimentarse de pradera, ensilado y forraje debido a la posibilidad de digerir los componentes de estos forrajes como celulosa y hemicelulosa, condición que los animales monogástricos no pueden realizar (Relling y Mattioli, 2003).

Según (Weimer, 1998), se debe agilizar el trabajo de la microbiota ruminal para la digestión de la fibra proveniente de los forrajes.

Cualquier alimento y agua que el animal consume es fermentado teniendo como resultado células microbianas, ácidos grasos volátiles y gases como dióxido de carbono y metano (McDonald *et al.,* 1995).

El animal y el rumen trabajan en conjunto ya que el primero suministra el alimento y el medio adecuado como anaerobiosis y pH para el desarrollo de bacterias que le darán a él la energía para su desarrollo y ciclo productivo (Ángeles, 2000).

2.2. PROCESO FERMENTATIVO

Los rumiantes tienen la capacidad de darle trasformación a la celulosa y la hemicelulosa que aseguran forman un aproximado del 70 % de la biomasa vegetal. (Ladisch, et al. 1990),

El tipo y número de microorganismos presentes en el rumen están directamente asociados con los ingredientes que contiene la dieta de los mismos (Febel y Fekete, 1996).

El resultado final de que los microorganismos lleven a cabo la fermentación es producir Ácidos Grasos Volátiles, acético, butírico, propiónico y láctico; mismos que serán la fuente nutricional para la actividad metabólica del rumiante, por lo cual el rendimiento de producción del animal está directamente relacionado con la actividad y calidad de la microbiota ruminal; además dela formación de otros compuestos como gases en su mayoría metano y dióxido de carbono (Roderick y White, 1990). La composición de los gases según (Calsamiglia y Ferret, 2002); es de 65% de CO_2, 27% de CH_4, siendo estos dos los mas importantes

2.4 Metano CH_4

El metano (CH_4) es un producto final de la fermentación que sufren los alimentos en el rumen, que en términos de energía constituye una pérdida y en términos ambientales contribuye al calentamiento y al cambio climático global. La investigación en nutrición animal se ha enfocado en su mayor parte a encontrar métodos para reducir las emisiones de CH_4 debido a la ineficiencia energética que ocurre en el rumen, y no por el rol del CH_4 en el calentamiento global. Sin embargo, últimamente se ha prestado más atención por la contribución potencial al cambio climático (Bonilla y Lemus 2012).

La producción de metano en los últimos años ha tomado gran importancia en la producción animal justamente por los efectos negativos en el medio ambiente (Chandramoni y Jadhao 2000).

Los bovinos poseen un sistema digestivo que tiene la capacidad de aprovechar y convertir material fibroso con altos contenidos de carbohidratos estructurales, en alimentos de alta calidad nutritiva, la carne y la leche. Sin embargo por sus características innatas, este mismo sistema digestivo también produce metano, un potente gas con efecto invernadero que contribuye con aproximadamente el 18% del calentamiento global ocasionado por actividades productivas con animales domésticos, superado sólo por el CO_2 (Montenegro y Abarca 2000).

La manipulación de la dieta de los rumiantes se considera una alternativa viable para aminorar la producción de metano y a la par disminuir las pérdidas energéticas en el animal (Carmona y Bolivar 2005).

(Johnson y Johnson 1995), señalan que las emisiones de gas metano por el ganado bovino, están estimadas en 58 millones de toneladas/ año, lo que representa el 73% del total de emisiones (80 millones) de todas la especies domésticas. (McCaughey 1999) indican que los animales domésticos, principalmente el ganado bovino son responsables de aproximadamente el15% de la producción de metano global.

2.5 ADICION DE LEVADURAS COMO ADITIVOS EN LA ALIMENTACION DE RUMIANTES

Los aditivos nutricionales han sido ampliamente utilizados y recomendados por nutricionistas, permitiendo un "ajuste fino" en las dietas. Uno de los principales aditivos utilizados en la nutrición de rumiantes es el cultivo de levadura. Este término genérico se refiere al producto que contiene células de levadura y el medio donde estas crecieron. Es de suma importancia considerar otras características que diferencian a los varios productos encontrados en el mercado, así como la viabilidad de células, especificidad de la cepa e idoneidad de la empresa, para que se pueda analizar y definir la tecnología que mejor se adapta en su realidad (Van Vuuren, 2003).

Tiempo atrás se inició con lo que recientemente se ha convertido en una interesante manera de trabajar con la situación alimentaria de los rumiantes, misma que ya no solo es con la formulación de dietas a base de forrajes y sus derivados, si no con la introducción de aditivos como son las levaduras que en los últimos años han pasado por procesos de evaluación para determinar si son económicamente rentables para aplicarlas y con ello incrementar considerablemente la productividad de los animales la mejora en la actividad digestiva (Yang 2001).

3. JUSTIFICACIÓN

La creciente demanda de productos de origen animal como lo son la carne, la leche y sus derivados a influido en el creciente desarrollo de la ganadería teniendo como efecto secundario el crecimiento exponencial de sus desechos orgánicos e inorgánicos entre los cuales se encuentran los gases de efecto invernadero lo cual a llevado a los nutricionistas del ramo en buscar alternativas para la disminución de los mismos , teniendo como efecto el desarrollo de diferentes alternativas como lo es la adicción de levaduras en las dietas del ganado por lo cual se busca el efecto directo de las mismas en las producción de gas metano CH_4

4. HIPÓTESIS

La adición de las levaduras en las dietas del ganado lechero y de engorda utilizadas en la alimentación del mismo posiblemente reducen la emisión del CH_4

5. OBJETIVO

Elaborar un meta-análisis del efecto de las levaduras sobre la emisión de CH_4 en el ganado lechero y ganado de engorda

6. MATERIALES Y MÉTODOS

6.1. Búsqueda de literatura

Se realizó una extensa búsqueda bibliográfica utilizando bases de datos de ISI Web of Knowledge (Thomson Reuters). El período de publicación de los estudios fue de enero de 1990 a diciembre de 2016. Las palabras clave utilizadas para la búsqueda de estudios relevantes incluyen (vaca lechera O carne de vaca) Y metano Y (levadura O *Saccharomyces cerevisiae*). Con el fin de asegurar la compilación de todos los estudios relevantes sobre meta análisis, se revisaron las referencias de los trabajos recopilados (Lean et al., 2009). En el caso de que los resultados experimentales se imprimieran en disertaciones, la disertación también se incluyó en la literatura revisada.

6.2. Los criterios de inclusión y exclusión

Después de la recopilación de los estudios publicados entre 1990 y 2016, los informes en ganado lechero y de carne de vacuno se analizaron para una selección posterior. Dichos estudios deben incluir tanto un grupo receptor de levadura (tratado) como un grupo control (no se administra levadura). Además, solo se incluyeron estudios con mediciones detalladas in vivo de la producción de CH_4. Los trabajos de revisión, así como los experimentos in vivo e in vitro sobre la influencia de la levadura en los parámetros de producción y la producción de otros animales CH_4 (ovejas y cabras) que se excluyeron de la literatura.

6.3. Extracción de datos

Los datos utilizados en el presente meta-análisis fueron aquellos para la producción diaria media de CH_4 o la emisión media de CH_4 por unidad de DMI (g / kg). Además, otros datos utilizados fueron SEM (error estándar de la media), SED (error estándar de diferencia) y número de vacas en receptores de levadura y grupos de control. También se registró otra información como el nombre del autor, el año de publicación, el método de medición de CH_4 y la raza de las vacas, el tipo de productos de levadura y la ración nutricional.

6.4. Análisis estadístico

El análisis estadístico se realizó utilizando el software completo de meta-análisis (versión 2.2). El tamaño del efecto para la producción diaria de CH_4 y CH_4 / DMI (g / kg) para todos los estudios incluidos (ganado lechero y ganado de carne) se determinó como la diferencia de medias estandarizada al 95% de los intervalos de confianza. Uno de los métodos utilizados para los datos continuos es la estimación de SMD (Lean et al., 2009). La DME es la diferencia media entre los grupos de tratamiento y control que está estandarizada en función de la desviación estándar (DE) de los grupos de tratamiento y control. El SMD permite la comparación de las diferencias entre los grupos en diversas variables (Borenstein et al., 2009). El modelo adoptado en este meta-análisis fue un modelo de efectos aleatorios (Borenstein et al., 2009).

La grafica de Forest es una de las gráficas comunes utilizadas en el meta-análisis que representa la información de cada estudio, así como el resultado final de todos los estudios (Lean et al., 2009). En este meta-análisis, la producción de CH_4 y el CH_4 / DMI (g / kg) de todos los estudios (ganado lechero y ganado de carne) se desarrollaron en una gráfica de Forest. En la gráfica de Forest, el tamaño del efecto es igual al DME con un intervalo de confianza del 95% en el caso de adoptar un modelo aleatorio.

6.5. Evaluación de la heterogeneidad

Con el fin de evaluar la heterogeneidad entre los estudios, se utilizaron la prueba Q y la estadística I^2 (Borenstein et al., 2009). Como el poder de la prueba Q en los estudios metaanalíticos con un bajo número de estudios es insignificante, se supuso que el nivel de significación era igual a 0.1. Aunque la prueba Q contribuye a la detección de la heterogeneidad, el valor cuantitativo (forma percentil) se determina a través de la estadística I^2. Si I^2 excede el 50 por ciento, se presumirá que los parámetros tienen una heterogeneidad significativa (Lean et al., 2009).

6.6. El sesgo de publicación

El sesgo de estudio (detección de posibles valores atípicos) se examinó a través de la gráfica Funnel y el método Trim & Fill (Duval y Tweedie, 2000). La grafica se refiere al índice estimado de cada estudio en comparación con su tamaño de muestra de precisión. Por lo tanto, un mayor tamaño del estudio se correlaciona con su mayor precisión; tales estudios están representados en la parte superior de la gráfica. Los estudios con menor tamaño están representados en la parte inferior de la gráfica (Lean et al., 2009). En el caso de que el gráfico Funnel muestre sesgo de uno o más de los estudios utilizados, el número de estudios que se excluirán y el tamaño del efecto ajustado se determinaron mediante el método de ajuste y relleno.

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ANEXO 1 ARTÍCULO CIENTÍFICO

Influence of *Saccharomyces cerevisiae* on the environmental efficiency of methane production in dairy and beef cattle: Meta-analysis

Abstract

The use of feed additives (e.g. yeasts products) for reduction of ruminants' methane (CH_4) production has drawn the attention of cattle nutritionists and environmentalists. Although there are many relevant studies on this topic, but the obtained results for dairy and beef cattle are contradictory. Therefore, the objective of the present study is to examine the effect of yeast (*Saccharomyces cerevisiae*) on reduction of CH_4 production in dairy and beef cattle using meta-analytic methods. After compilation of relevant articles published 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 effect size of yeast on CH_4 production (g/day) and CH_4 production per dry matter intake (CH_4/DMI (g/kg)) was estimated as standardized mean difference at 95% confidence interval. In addition, heterogeneity across studies was tested by using Q test and

I^2 index. Study bias was verified through Funnel plot and trim-and-fill method. The results of meta-analysis of all 3 groups of animals (all cattle, dairy cattle, and beef cattle) suggested that effect size of yeast on CH_4 production (g/day) and CH_4/DMI (g/kg) was not significant. In other words, the use of yeast for dairy and beef cattle did not reduce CH_4 production and CH_4/DMI (g/kg) significantly. The results of Q test and I^2 index suggest that there is no heterogeneity between different studies on CH_4 production and CH_4/DMI (g/kg) so that value of I^2 index of CH_4/DMI (g/kg) in dairy and beef cattle was about 14% while for dairy cattle, the index is about 40%. There was no study bias of CH_4 production as well as CH_4/DMI (g/kg). As shown in funnel plot, the studies are more concentrated in the lowest part of the plot. 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 CH_4 production in dairy and beef cattle. It is recommended to conduct further studies on effect of different doses of yeast, use of yeast products, different strains and experimental designs.

Keywords: Yeast, Meta-analysis, Methane, Dairy Cattle, Beef Cattle.

1. Introduction

Undoubtedly, one of the significant sources of greenhouse gas emissions is livestock. Among the greenhouse gases, CH_4 is one of the major gases produced out of enteric fermentation in ruminants and it has 25 times more potential of global warming than CO_2. Beside of adverse effects that CH_4 exerts 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 CH_4 (Johnson and Johnson, 1995). The reticulorumen is a fermentation chamber containing a complex and diverse microbiota composed of different microbial communities such as bacteria, archaea, protozoa, fungi, and phages all of which turn the rumen into a place for microbial fermentation of feed. As a by-product of fermentation, hydrogen molecules could be reduced by methanogens into methane and CO_2. Therefore, in past few years' numerous studies have been conducted concerning possible reduction of CH_4 production per unit of ruminants' meat and milk. This has led to development of numerous strategies for reduction of ruminants' CH_4 production. In terms of ruminants' nutrition, some solutions such as modification of concentrate, type and quality of forage, defaunation, and use of ionophores, oils, organic acids, direct fed microbial and prebiotics are suggested (Boadi et al., 2004; Iqbal et al., 2008).

One of the solutions for reduction of ruminants' CH_4 production which has drawn a considerable attention in recent years is use of yeasts, as one type of direct fed microbes or probiotics. Before use of yeast products as a probable solution for reducing production of CH_4, such products were used as feed additive for ruminants which improved production performance (increase of growth rate, meat and milk) and inhibited acidosis thus improving animal health and welfare (Chaucheyras-Durand et al., 2008; Vohra et al., 2016). As a natural feed additive, yeasts contribute to balance and stabilize rumen microbiota, to maintain a favorable pH in rumen and formation of fermentation end-products, and to improve ammonia utilization by ruminal bacteria. The previous meta-analytic studies on effect of yeast on ruminants (Desnoyers et al. 2009) and 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 meta-analytic study of

Sartori et al. (2017) suggested that adding yeast to ration of beef cattle could reduce dry matter intake but exerts no effect 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 effect of yeast products on reduction of CH_4 production of dairy and beef cattle. The review of results on the association between CH_4 production and CH_4 emission intensity (CH_4/DMI (g/kg)) show some controversial outcomes. Regarding dairy cattle, Muñoz et al. (2017) 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 CH_4 production. However, the studies conducted by Bayat et al. (2015) and Chung et al. (2011) suggested that yeast-recipient group had lower CH_4 production, although the difference from control group was insignificant. One of the supplementation strains used in the feed of beef cattle was suggested by McGinn et al. (2004) as reducing CH_4 production in comparison with control group but the difference between CH_4 productions of both groups was insignificant. Regarding the results of CH_4 emission intensity, Muñoz et al. (2017) reported that addition of yeast was followed by higher yield of CH_4 per kg dry matter intake and digestible OM intake. In contrast, Chung et al. (2011) found out that one of the strains of *Saccharomyces cerevisiae* tended to cause a relative reduction of CH_4 production when compared with other strain.

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 feedcould 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 (Yea-Saac, 1026) reduces methane production up to 10% in 12 hours when a barley-based beef ration was used.

In general, the use of yeast products as an additive to dairy and beef cattle rations could improve performance. However, CH_4 mitigation by yeast could be a relatively ambiguous and contradictory matter (Hristov et al., 2013). Although *in vitro* studies are also regarded as a valuable way of testing ideas, *in vivo* tests could offer real-world results. Therefore, in this meta-analysis, only data from *in vivo* reports were reviewed and used. Since meta-analysis could summarize results of different studies into statistical information, the objective of present paper is to survey the influence of yeast (*Saccharomyces cerevisiae*) on CH_4 production as well as CH_4/DMI (g/kg) in dairy and beef cattle through meta-analytical methods.

2. Material and methods

2.1. *Literature search*

An extensive literature search was carried out using databases of ISI Web of Knowledge (Thomson Reuters). The publication period of studies was from January 1990 to December 2016. The keywords used for search of relevant studies include (dairy cow OR beef) AND methane AND (yeast OR *Saccharomyces cerevisiae*). In order to assure the compilation of all relevant studies on meta-analysis, references of collected papers were reviewed (Lean et al., 2009). In the case that experimental results were printed in dissertations, the dissertation was also included in the reviewed literature.

2.2. *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 group (no yeast administered). In addition, only studies with detailed *in-vivo* measurement of CH_4 production were included. The review papers as well as *in-vivo* and *in vitro* experiments on influence of yeast on production parameters and CH_4 production of other animals (sheep and goat) were excluded from the literature.

2.3. *Data Extraction*

Data used in present meta-analysis were those for mean daily CH_4 production or mean CH_4 emission per unit of DMI (g/kg). In addition, other data used were SEM (standard error of the mean), SED (standard error of difference), and number of cows in yeast-recipient and control groups. Other information such as name of author, year of publication, CH_4 measurement method, and breed of cows, type of yeast products, and nutritional ration was also recorded.

2.4. *Statistical Analysis*

Statistical analysis was done using Comprehensive Meta-Analysis Software (version 2.2). The effect size for daily CH_4 production and CH_4/DMI (g/kg) 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. The SMD enable comparison of differences between groups in diverse variables (Borenstein et al., 2009). The model adopted in this meta-analysis was a random effects model (Borenstein et al., 2009).

Forest plot is one of the common plots used in meta-analysis which represents information of each study as well as final outcome of all the studies (Lean et al., 2009). In this meta-analysis, CH_4 production and CH_4/DMI (g/kg) 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.

2.5. *Assessment of Heterogeneity*

In order to test heterogeneity across studies, Q test and I^2 statistic were used (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 with 0.1. Although Q-test contributes to detection of heterogeneity, the quantitative value (percentile form) is determined through I^2 statistic. If I^2 exceeds 50 percent, the parameters will be presumed to have significant heterogeneity (Lean et al., 2009).

2.6. *Publication Bias*

Study bias (detection of possible outliers) was examined through Funnel plot and Trim & 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.

3. Results

3.1. *Characteristics of the Database*

The list of collected studies as well as data used in the present meta-analysis are represented in table 1. 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. One should note that the results lack high statistical power. In some experiments on dairy cattle (e.g., Bayat et al., (2015); Chung et al., (2011)) 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 CH_4 through SF6 method. Other studies measured level of CH_4 using respiratory chambers. Among previous experiments of dairy cattle, Chung et al. (2011) conducted experiments on non-lactating cows. In other experiments of dairy cattle, the cows were in lactation. Among experiments on beef cattle, McGinn et al. (2004) conducted a test on growing beef cattle while Possenti et al. (2008) used cross-bred cows. Meller (2016) and Hristov et al. (2010) used live yeast culture but the rest of experiments were conducted through active dried yeast. In all of the experiments included in present meta-analysis, the yeast used was *Saccharomyces cerevisiae*.

3.2. *CH_4 Production*

The results of the meta-analysis of the effect of yeast on CH_4 production by dairy and beef cattle are represented in table 2. The meta-analytic results of 11 comparisons between yeast-recipient group and control group suggested that yeast does not exert significant effect on reduction of CH_4 production when dairy and beef cattle were pooled (SMD= -0.031; P=0.875). The difference between control and yeast groups were not significant for dairy cows (SMD= -0.056; P=0.800) or for beef cattle (SMD=0.057; P=0.889). As shown in table 2, the Q test and I^2-statistic showed that there was no heterogeneity across studies on CH_4 production. The Q value for CH_4 production in dairy and beef cattle, dairy cattle and beef cattle was more than 0.1 and I^2 index was zero.

The forest plot of CH_4 production (Fig. 1) showed final outcome as SMD. In the plot, each comparison between yeast-receiving group and control group is represented by a square and confidence level 95 percent is shown by a transverse line.

The results of publication bias obtained through review of Funnel plot (Fig. 3) and trim and fill method suggest that there is no publication bias in terms of CH_4 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.

3.3. CH_4/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 yeast does not exert significant effect on reduction of CH_4/DMI in pooled dairy and beef cattle (SMD= -0.087; P=0.722), only dairy cattle (SMD= -0.120; P=0.732), or only beef cattle (SMD=0.002; P=0.996). No significant heterogeneity was found across studies on CH_4/DMI The p-value in the Q test was higher than 0.1. Although value of I^2 for dairy cattle was about 40 percent, the index is zero for beef cattle. The forest plot (Fig. 2) of CH_4/DMI shows the final outcome as SMD.

The results of publication bias (Fig. 4) of CH_4/DMI of dairy and beef cattle suggest that there is no publication bias. Similar to previous plot (Fig. 3), the studies are clustered at the bottom of the plot.

4. 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 studies and meta-analytic studies on effect of using yeast in 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 and concentration of volatile fatty acids in rumen but reduces concentration of lactic acid in rumen and exerts no effect on acetate to propionate ratio in ruminants. In addition, supplementation of yeast increases DMI, milk production and milk fat without influencing protein content of the milk. In a meta-analysis 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 meta-analysis 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 CH_4 production. Some of these studies are shown in table 1. The results of present meta-analysis suggest that adding yeast to ration of dairy and beef cattle did not reduce CH_4 production. The experiments of Bayat et al. (2015) and Chung et al. (2011) on dairy cattle suggested that CH_4 production (g/day) of yeast-receiving cows is less than in control cows. McGinn et al. (2004) conducted a similar test on beef cattle and found out that only one yeast-receiving group had produced lower CH_4 than the control group. In other experiments, no reduction of CH_4 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) easily. In the forest plot (Fig. 1), 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 meta-analysis suggest that supplementation of yeast did not reduce of CH_4/DMI. The experiments of Bayat et al. (2015), Chung et al. (2011), and Meller (2016) on dairy cattle as well as the experiment by McGinn et al. (2004) on beef cattle suggest that only one yeast-receiving group showed lower CH_4 production per kilogram of dry matter intake than control the group. The forest plot (Fig. 2) 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 CH_4 production significantly, the result of *in-vivo* experiments in sheep suggested that *Trichosporom sericoum* yeast culture reduced daily CH_4 production to a greater extent than control group (33.39 vs. 37.18 l/day). However, no significant reduction of CH_4/DMI (g/kg) 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 CH_4 production per dry matter intake (Lu et al., 2016). It seems that in sheep and goats the influence of yeast on reduction of CH_4 production could be more positive. Although further tests on sheep and goat is required to confirm this inference.

Another important parameter related to methane production in the rumen is the acetate: propionate ratio. Moss et al. (2000) suggested that while acetate and butyrate formation stimulate methane production, e propionate could act as an alternative pathway for hydrogen intake in 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 ratio of VFAs so that the proportion of acetate is decreased whereas that of propionate increases. S*accharomyces 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 withdaily CH_4 production. In the case of CH_4/DMI, the I^2 index 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. The differences among experiments on the effect of yeast on CH_4 production and CH_4/DMI might be due to diverse factors such as strain of yeast, viability, feed intake and/or management (Chaucheyras-Durand et al., 2008). The comparative study with two yeast strains versus a control group did not report a significant difference between strains in CH_4 production. Bayat et al. (2015), Chung et al. (2011) and McGinn et al. (2004) used two strains of yeast. Their findings suggested that CH_4 production (g/day) and amount of CH_4 per dry matter intake (g/kg) did not show a significant difference between treatment cows with none of the strains. Regarding the type of yeast product, two studies used yeast culture. The cows receiving yeast culture did not show a significant decline in CH_4 production or CH_4/DMI in comparison with the control group (Meller, 2016). Elghandour et al. (2017) conducted an in-vitro test 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 above-mentioned cases, the type of experimental design might also affect the results. In most of the tests, a cross-over design was used. In the present meta-analysis, the experiment conducted by Chung et al. (2011) was based on randomized block design but the rest of the tests were done based on cross-over design. Such design may be improper for examining the effect of yeast on rumen-related parameters since yeasts could be influential upon equilibrium of microbial population (Chaucheyras-Durand et al., 2012). In addition, Bayat et al. (2015) conducted a study on the effect of two strains of yeast on CH_4 production in dairy cattle and suggested that a slight reduction of CH_4 production occurred in yeast-receiving treatments. The decrease was not significant and this might have been due to low number of observations.

The technique used in measurement of produced CH_4 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 stature (i.e. physiological step) of the animal might be also influential upon results. As a result, 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 startegy for reduction of methane.

5. 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 CH_4 production and CH_4/DMI. In addition, no significant heterogeneity was observed between different 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 observed.

Conflict of interest

None declared.

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