UNIVERSIDAD AUTÓNOMA DEL ESTADO DE MÉXICO
MAESTRÍA Y DOCTORADO EN CIENCIAS
AGROPECUARIAS Y RECURSOS NATURALES

Saccharomyces cerevisiae Y SU IMPACTO SOBRE LA CAPACIDAD FERMENTATIVA MICROBIANA EN HERBÍVOROS

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QUE PARA OBTENER EL GRADO DE
DOCTORA EN CIENCIAS AGROPECUARIAS Y RECURSOS NATURALES

P R E S E N T A :

M en C. MONA MOHAMED MOHAMED YASSEEN ELGHANDOUR

El Cerrillo Piedras Blancas, Toluca, Estado de México, Febrero de 2016

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RESUMEN

La incorporación de microorganismos directamente a la alimentación, ofrece un gran potencial para la manipulación de la fermentación ruminal y *Saccharomyces cerevisiae* es un organismo especialmente atractivo. Se ha reportado que la incorporación de *S. cerevisiae* aumenta el valor nutricional de los forrajes de baja calidad. La presente tesis de investigación, en tres experimentos, tuvo como objetivo evaluar el impacto de *Saccharomyces cerevisiae* sobre el valor nutritivo de algunos forrajes de baja calidad, así como también sobre raciones integrales con diferente concentración de proteína cruda en la alimentación animal (rumiantes y equinos). Los resultados de los tres experimentos fueron publicados en revistas científicas indexadas con factor de impacto.

Del primer experimento, sus resultados fueron publicados como un trabajo de investigación original en el **Journal of Equine Veterinary Science 34 (2014), 619-625**, y tuvo como objetivo evaluar *in vitro* la capacidad fermentativa de inoculos fecales equinos sobre nueve forrajes fibrosos en presencia de *Saccharomyces cerevisiae*. Los alimentos fibrosos fueron: rastrojo de maíz (*Zea mays*), paja de avena (*Avena sativa*), bagazo y hojas de caña de azúcar (*Saccharum officinarum*), hojas de pasto llanero (*Andropogon gayanus*), hojas de pasto Taiwán (*Pennisetum purpureum*), paja de sorgo (*Sorghum vulgare*) y hojas de pasto estrella (*Cyinodon plectostachyus*). Las Muestras de piensos fibrosos se incubaron con varias dosis de *S. cerevisiae*; 0 (control), 1.25 (baja), 2.5 mg (medio) y 5.0 (alto) mg / g de MS de un producto de levadura comercial que contiene 1×10^{10} UFC/ gramo. El inóculo fecal se obtuvo de cuatro caballos adultos alimentado *ad libitum* con un concentrado comercial que contiene heno de alfalfa. La producción de gas (PG) se registró a las 2, 4, 6, 8, 10, 12, 24 y 48 h después de la inoculación. Se observó una interacción entre los alimentos y la dosis de levadura para el pH fecal ($P < 0.01$), la asintótica de PG (*b*, ml / g MS); tasa de PG (*c*, / h); retraso inicial previo a la PG (*L*, h), PG en 4 h y 48 h ($P < 0.01$), PG a las 8 h ($p < 0.01$), y a las 24 h ($P < 0.01$). Diferencias en la capacidad de fermentación fecal entre los forrajes tropicales y templados ($P < 0,05$) ocurrieron para el pH fecal, *c* y PG durante las primeras 12 horas, así mismo, se presentaron

diferencias (P <0,05) entre los subproductos de la agricultura y los pastos para el pH fecal, *b*, y PG a partir de 8 a 48 h. La capacidad de fermentación entre los forrajes fibrosos frente a los no fibrosos (P <0,05) difirió para el pH fecal, *b*, y PG después de 12 h. La adición de *S. cerevisiae* al rastrojo de *Z. mays* reduce (P <0.01) el pH fecal y la fracción *c* con una mayor (P <0.01) fracción *b* en comparación con los otros alimentos. A partir de 4 a 24 horas, el bagazo de *S. officinarum* aumento la PG a los valores más altos en comparación con las hojas *de S. officinarum*. Después de 24 h, el rastrojo de *Z. mays* tuvo la más alta PG, mientras que las hojas de *C. plectostachyus* fue la más baja PG. No hubo diferencias entre las dosis de levadura para todos los parámetros medidos con la excepción de los valores de *L* (efecto lineal; P <0,01). El rastrojo de *Z. mays* tuvo el mayor valor nutritivo en comparación con los otros alimentos fibrosos. Sin embargo, la adición de *S. cerevisiae* en 2,5 a 5,0 g / kg MS ha mejorado la capacidad de fermentación fecal de forrajes de baja calidad.

Los resultados del segundo experimento fueron publicados en la revista **Italian Journal of Animal Science 13 (2014), 295-301** y evaluaron los efectos de *Saccharomyces cerevisiae* en la producción de gas *in vitro* (PG) y la degradabilidad de rastrojo de maíz, paja de avena, bagazo de caña y paja de sorgo. Los alimentos se incubaron con diferentes dosis de levadura (0, 4, 8 y 12 mg / g de MS) en adición directa o con 72 h de pre-incubación. La PG fue registrada a las 2, 4, 6, 8, 10, 12, 14, 24, 30, 48, 54 y 72 h de incubación. Después de 72 h, el pH y el metano se determinaron y los residuos se filtraron para determinar la degradabilidad de MS, fibra detergente neutro (FDN) y fibra detergente ácido (FDA). Existieron interacciones (P <0.001) entre las especies fibrosos × el método de aplicación × los niveles de levadura para todos los parámetros medidos de PG y degradabilidad ruminal. La adición directa o con 72 h de pre-incubación de *S. cerevisiae* al rastrojo de maíz mejoró (P <0.05) la PG y la producción de metano y redujo (P <0.05) el tiempo de retraso para el inicio de la fermentacón (*L*) y la degradabilidad de la FDN (DFDN). La adición directa de *S. cerevisiae* a la paja de avena aumentó (P <0.05) la tasa de PG (*c*) y disminuyó (P <0.05) la asintótica de PG (*b*). Sin embargo, la pre-incubación de 72 h aumentó (P <0.05) la tasa *c* una disminución lineal de *b*, de la degradabilidad de la MS (DMS) y DFDN. La aplicación de *S. cerevisiae* durante 72 h pre-incubación

disminuyó (P <0.001) las emisiones de metano. La adición directa o la pre-incubación a 72 h con *S. cerevisiae* a la paja de sorgo aumentó (P <0.05), las fracciones *b, c, L*, así como la DMS y DFDN. En general, el efecto de la dosis varió entre los diferentes piensos y diferentes métodos de aplicación. Los resultados sugieren que la adición directa de 4 a 12 g de *S. cerevisiae* / kg MS puede mejorar la fermentación ruminal de los forrajes de baja calidad.

El tercer experimento de investigación ha sido aceptado para su publicación en el **Journal of Applied Animal Research – (ID JAAR-2015-0012)** y su objetivo fue evaluar si el efecto de *Saccharomyces cerevisiae* (SC) sobre la fermentación *in vitro* puede ser afectado por el contenido de proteína cruda (PC) de la ración. Se probaron tres cultivos comerciales de *Saccharomyces cerevisiae* (SC): Biocell F53®, Procreatin 7® y Biosaf SC47® para evaluar niveles de 0 (SC0), 2 (SC2) y 4 (SC4) mg / g de MS de sustrato. Dos raciones niveles bajo 13% (BPC) y alto 16% de PC (APC) fueron utilizados como sustratos. La producción de Gas (GP) y metano (CH4) se registraron a las 2, 4, 6, 8, 10, 12, 14, 24, y 48 h de incubación. No se observaron interacciones (P <0.05) entre la ración × cultivo de levadura × dosis de levadura para PG, producción de CH4, y la cinética de la fermentación. La ración APC aumentó (P = 0.05) la asintótica de PG, la producción de CH4, y los parámetros de fermentación. Biocell F53® y Biosaf SC47® aumentaron la asintótica de PG (P <0.05) en las raciones de APC y BPC con un mejor efecto a la dosis de 2 mg / g MS de sustrato para APC (p <0,05) y dosis de 4 mg de levadura/ g MS de sustrato para BPC. Se observó la mayor producción de CH4 (P <0.05) con Procreatin 7®. Por otra parte, Procreatin 7® a 2 mg / g MS mejoró (P <0.05) la cinética de fermentación de la ración APC que las otras dosis de los otros cultivos de levadura, mientras que con la ración BPC, la dosis de 2 mg / g de MS de Biocell F53 ® tuvo la mejor cinética de fermentación (P <0.05). Podría concluirse que la ración APC mejoró la PG comparada con la ración BPC. Por otra parte, la adición de Biocell F53® y Biosaf SC47® a un nivel de 2 mg / g de MS mejora la cinética de fermentación y la degradabilidad de los nutrientes.

Como conclusión general, la adición de *S. cerevisiae* puede aumentar la fermentación ruminal de forrajes de baja calidad mejorando la cinética de fermentación ruminal y la producción de gas, así como también, reduciendo la producción de metano. Las raciones con alto contenido de proteína cruda produjeron mayor cantidad de gas y disminuyeron la producción de metano en comparación con las dietas de bajo contenido en proteína cruda. El efecto de *S. cerevisiae* sobre la fermentación de alimentos depende de la composición química de estos, especialmente su contenido en fibra y proteína cruda.

Palabras clave: Degradabilidad; Inóculo fecal equino; Alimentos fibrosos, Producción de gas *in vitro*; Metano; Nivel de proteína; Fermentación ruminal; Levadura.

ABSTRACT

Direct-fed microbial offer a great potential for manipulation of ruminal fermentation and *Saccharomyces cerevisiae* is an especially attractive organism. *S. cerevisiae* addition was reported to increase nutritional value of poor quality forages. The present research work of this thesis was aimed to evaluate the impact of *Saccharomyces cerevisiae* on the nutritive value of some poor quality forages as well as total mixed rations with different crude protein concentrations in animal nutrition (ruminates and horses) in three experiments. The results of the three experiments were published in three indexed scientific journals with impact factor.

The **first experiment** results were published as an original research paper in the **Journal of Equine Veterinary Science 34 (2014), 619–625** and aimed to evaluate the *in vitro* effects of equine fecal inocula fermentative capacity on nine fibrous forages in the presence of *Saccharomyces cerevisiae*. The fibrous feeds were corn stover (*Zea mays*), oat straw (*Avena sativa*), sugarcane bagasse and leaves (*Saccharum officinarum*), *llanero grass leaves (Andropogon gayanus),* taiwan grass leaves (*Pennisetum purpureum*), sorghum straw (*Sorghum vulgare*) and steria grass leaves (*Cyinodon plectostachyus*). Fibrous feed samples were incubated with several doses of *S. cerevisiae*; 0 (control), 1.25 (low), 2.5 mg (medium) and 5 (high) mg/g DM of a commercial yeast product containing 1×10^{10}/gram. Fecal inoculum was collected from four adult horses fed *ad libitum* a commercial concentrate containing alfalfa hay. Gas production (GP) was recorded at 2, 4, 6, 8, 10, 12, 24 and 48 h post inoculation. An interaction occurred between feeds and yeast dose for fecal pH (P<0.01), asymptotic GP (b, ml/g DM); rate of GP (c, /h); initial delay before GP begins (L, h), GP at 4h and 48h (P<0.01), GP at 8 h (P<0.01), and at 24 h (P<0.01). Differences in fecal fermentation capacity between the tropical and template grass (P<0.05) occurred for fecal pH, c and GP during first 12 h, whereas differences occurred (P<0.05) between the agriculture by-products and the grasses for fecal pH, b, and GP from 8 to 48 h. Fermentation capacity between straws *versus* not straws (P<0.05) differed for fecal pH, b, and GP after 12 h between straws *versus* not straws. Addition of *S. cerevisiae* to *Z. mays* stover reduced (P<0.01) fecal pH and the c fraction with a higher (P<0.01)

b fraction *versus* the other feeds. From 4 to 24h, *S. officinarum* bagasse improved GP to the highest values *versus S. officinarum* leaves. After 24 h, *Z. mays* stover had the highest GP, while *C. plectostachyus* leaves had the lowest. There were no differences among the yeast doses for all measured parameters with the exception of *L* values (linear effect; $P<0.01$). The *Z. mays* stover had the highest nutritive value compared to the other fibrous feeds. However, addition of *S. cerevisiae* at 2.5 to 5.0 g/kg DM improved fecal fermentation capacity of low quality forages.

The **second experiment** results were published in the **Italian Journal of Animal Science 13 (2014), 295-301** and carried out to evaluate the effects of *Saccharomyces cerevisiae* on *in vitro* gas production (GP) and degradability of corn stover, oat straw, sugarcane bagasse and sorghum straw. Feedstuffs were incubated with different doses of yeast (0, 4, 8 and 12 mg /g DM) at direct addition or 72h pre-incubation. Rumen GP was recorded at 2, 4, 6, 8, 10, 12, 14, 24, 30, 48, 54 and 72h of incubation. After 72h, the rumen pH and methane were determined and contents were filtrated for DM, neutral detergent fiber (NDF) and acid detergent fiber (ADF) degradability. Fibrous species × method of application × yeast interactions occurred ($P<0.001$) for all measured ruminal GP parameters and degradability. The direct addition or 72h pre-incubation of *S. cerevisiae* with corn stover improved ($P<0.05$) GP and methane and decreased ($P<0.05$) the lag time (*L*) and NDF degradability (NDFD). The direct addition of *S. cerevisiae* to oat straw increased ($P<0.05$) rate of GP (*c*) and decreased ($P<0.05$) asymptotic GP (*b*). However, 72h pre-incubation increased ($P<0.05$) *c* with linearly decreased *b*, DM degradability (DMD) and NDFD. Applying *S. cerevisiae* for 72h pre-incubation decreased ($P<0.001$) methane emission. The direct addition or 72h pre-incubation of *S. cerevisiae* to sorghum straw increased ($P<0.05$) the *b*, *c*, *L*, DMD and NDFD. Overall, the effect of dose varied among different feedstuffs and different application methods. Results suggested that the direct addition of *S. cerevisiae* could support and improve ruminal fermentation of low-quality forages at 4 to 12 g/kg DM.

The **third experiment** research work was accepted for publication in the **Journal of Applied Animal Research - ID JAAR-2015-0012** and aimed to evaluate if the

effect of *Saccharomyces cerevisiae* (SC) on *in vitro* fermentation can be affected with the crude protein (CP) content of the ration. The reaserch work included three commercial *Saccharomyces cerevisiae* (SC) cultures of Biocell F53®, Procreatin 7® and Biosaf SC47® were evaluated at 0 (SC0), 2 (SC2), and 4 (SC4) mg/g DM of substrate. *Two rations with 13% (LCP) and 16% CP (HCP) were used as substrates.* Rumen gas (GP) and methane (CH_4) productions were recorded at 2, 4, 6, 8, 10, 12, 14, 24, and 48 h of incubation. Interactions were observed (P<0.05) between ration × yeast culture × yeast dose for GP, CH_4 production, and fermentation kinetic parameters. The HCP ration had increased (P=0.05) asymptotic GP, CH_4 production, and fermentation parameters. Biocell F53® and Biosaf SC47® increased the asymptotic GP (P<0.05) in HCP and LCP rations with better effect for the dose of 2 mg/g DM substrate HCP (P<0.05) and dose of 4 mg yeast/g DM substrate with the LCP ration. The highest CH_4 production was observed (P<0.05) with Procreatin 7®. Moreover, Procreatin 7® at 2 mg/g DM had improved (P<0.05) fermentation kinetics of the HCP ration than other doses of other yeast cultures, while with the LCP ration, the dose of 2 mg/g DM from the Biocell F53® had better fermentation kinetics (P<0.05). It could be concluded that HCP ration improved GP than LCP ration. Moreover, addition of Biocell F53® and Biosaf SC47® at rate of 2 mg/g DM improved fermentation kinetics and nutrients degradability.

Key words: Degradability, Equine faecal inoculum, Fibrous feeds, Gas production; Methane, Protein level, Ruminal fermentation, Yeast.

I.- INTRODUCCIÓN

Los principales objetivos de los investigadores en el área de nutrición animal son: Mejorar la utilización de alimento, la salud, la producción animal y la seguridad alimentaria. Se han seguido algunas estrategias para lograr la fermentación deseable, reducir al mínimo los trastornos ruminales y excluir a agentes patógenos. Los aditivos alimenticios tales como antibióticos, ionóforos, inhibidores de metano, agentes defaunadores, enzimas exógenas, etc., han sido utilizados para manipular el ecosistema microbiano y la cinética de fermentación (Salem *et al.* 2015, Valdes *et al.* 2015). Sin embargo, el uso de estos ha presentado impactos negativos en la salud pública como la presencia de residuos químicos de aditivos en leche y carne, así como la resistencia bacteriana a antibióticos, por lo cual se han considerado algunas restricciones para su uso (Barton 2000). Es por esto que las investigaciones se han reorientado a la búsqueda de alternativas que reduzcan el impacto negativo de los aditivos del alimento sobre la salud pública, la salud animal y el ambiente.

Los cultivos de levadura son uno de los aditivos alimentarios más utilizados, mejoran la cinética de fermentación y la utilización del alimento, casi sin efectos tóxicos sobre los animales. El *Saccharomyces cerevisiae* tiene la capacidad de aumentar la tasa de degradación inicial y la digestión total de las fibras (Salem *et al.* 2015). Por otro lado, Kumar *et al.* (2013) y Pinloche *et al.* (2013) reportaron que la adición de levadura en la dieta tiene efectos positivos en las actividades microbianas y el ecosistema ruminal.

Se ha observado que la suplementación en la dieta con *S. cerevisiae* incrementa la proporción de bacterias anaerobias totales y celulolíticas (Newbold *et al.* 1996; Jouany 2001), lo que proporciona al rumen los nutrientes importantes y cofactores nutricionales requeridos para el crecimiento y actividad microbiana (Callaway y Martin 1997; Mao *et al.* 2013). Los cultivos de levadura contienen proporciones variables de células vivas y muertas de *S. cerevisiae;* que, dependiendo del número de células vivas o metabólicamente activas, causan diferentes respuestas en la alimentación de los animales (Salem *et al.* 2015).

El modo de acción de las levaduras para mejorar la fermentación y la utilización de alimentos depende de varios factores como: dosis, horarios y frecuencia de alimentación así como la cepa de levadura. Algunas cepas actúan dentro del rumen, mientras otras cepas tienen efecto en el tracto gastrointestinal. El modo de

acción puede ser explicado basado en varios mecanismos, incluyendo un efecto amortiguador de pH, y un mejor aprovechamiento del lactato (Martin y Streeter 1995). Las levaduras pueden ayudar a mantener la anaerobiosis en rumen, porque eliminan el oxígeno de las superficies del alimento recién ingerido (Newbold *et al.* 1996). Además, las levaduras tienen la capacidad de disminuir el potencial redox en el rumen (Jouany *et al.* 1999) y proporcionan mejores condiciones para el crecimiento de bacterias celulolíticas, anaerobias estrictas y estimulan su adhesión a las partículas de forraje (Roger *et al.* 1990). Las levaduras pueden mejorar las condiciones ruminales incrementando la tasa inicial de actividad celulolítica y competir con otras bacterias amilolíticas (Lynch y Martin 2002) resultando en la prevención de acumulación de lactato en rumen.

El efecto de la adición de levaduras en dietas para becerros pre-rumiantes tiene resultados prometedores. La levadura tiene un efecto positivo para modificar la función del tracto gastrointestinal a través de acelerar el establecimiento de los microorganismos ruminales e intestinales y evitar el establecimiento de enteropatógenos.

En vacas lecheras, la levadura puede ser útil sobre todo durante la lactancia temprana para prevenir la acidosis ruminal resultante de la alimentación de dietas con carbohidratos altamente fermentables (Kung 2006). Las levaduras de *Saccharomyces cerevisae* estimulan el crecimiento de otros microorganismos proporcionando metabolitos esenciales como propionato, aminoácidos y vitaminas; además de utilizar ciertos metabolitos bacterianos como fuente de carbono (Jespersen, 2003). Por otra parte, el aumento de la ingesta de materia seca, la producción y la composición de la leche, son otros beneficios de la alimentación con la levadura (Bruno *et al.,* 2009).

En producción de carne, es importante prevenir la acidosis ruminal resultante de la alimentación con dietas altamente fermentables. Como beneficios, se ha reportado que mejora el desempeño productivo, la producción de carne y la eficiencia alimenticia en muchos experimentos en los que se adicionó levadura a la dieta (Issakowicz *et al.* 2013).

Si bien se ha demostrado un efecto positivo en el uso de levaduras, las respuestas con *S. cerevisiae* dependen del tipo y composición del alimento, de los métodos de aplicación del aditivo, de las dosis de la levadura y de la interacción con

la dieta (Patra 2012). Por ello es necesario determinar las dosis a las cuales la levadura tiene mayor impacto sobre la digestibilidad de nutrientes y en el comportamiento productivo.

II.- REVISIÓN DE LITERATURA

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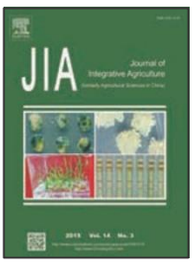

REVIEW

Direct-fed microbes: A tool for improving the utilization of low quality roughages in ruminants

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Abstract

For many years, ruminant nutritionists and microbiologists have been interested in manipulating the microbial ecosystem of the rumen to improve production efficiency of different ruminant species. Removal and restriction of antibiotics sub-therapeutic uses from ruminant diets has amplified interest in improving nutrient utilization and animal performance and search for more safe alternatives. Some bacterial and fungal microorganisms as a direct-fed microbial (DFM) can be the most suitable solutions. Microorganisms that are commonly used in DFM for ruminants may be classified mainly as lactic acid producing bacteria (LAB), lactic acid utilizing bacteria (LUB), or other microorganism's species like *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus*, *Propionibacterium*, *Megasphaera elsdenii* and *Prevotellabryantii*, in addition to some fungal species of yeast such as *Saccharomyces* and *Aspergillus*. A definitive mode of action for bacterial or fungal DFM has not been established; although a variety of mechanisms have been suggested. Bacterial DFM potentially moderate rumen conditions, and improve weight gain and feed efficiency. Fungal DFM may reduce harmful oxygen from the rumen, prevent excess lactate production, increase feed digestibility, and alter rumen fermentation patterns. DFM may also compete with and inhibit the growth of pathogens, immune system modulation, and modulate microbial balance in the gastrointestinal tract. Improved dry matter intake, milk yield, fat corrected milk yield and milk fat content were obtained with DFM administration. However, the response to DFM is not constant; depending on dosages, feeding times and frequencies, and strains of DFM. Nonetheless, recent studies have supported the positive effects of DFM on ruminant performance.

Keywords: direct-fed microbial (DFM), mode of action, ruminants

1. Introduction

The main goals of rumen microbial studies are to improve feed utilization, animal production and health, and animal food safety, which may be achieved by facilitating desirable fermentation, minimizing ruminal disorders, and excluding pathogens. For the past few decades, a number of chemical feed additives such as antibiotics, ionophores, methane inhibitors and defaunating agents have been used in rumi-

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nant nutrition to manipulate the microbial ecosystem and fermentation characteristics in the rumen and intestinal tract of livestock (Seo *et al.* 2010). Due to probable toxicity problems to the host animals, these feed additives are not routinely used (Salem *et al*. 2014a, b). Recently, a great awareness from public health aspects such as residues of these chemicals in milk and meat, and bacterial resistance to antibiotics as a result of increased use in the food chains prohibits their use as feed additives (Barton 2000). These supplements have been criticized by the consumers' organizations on the ground of product safety and quality. The consumers' demands have stimulated to search for natural alternatives to chemical feed additives. Supplementation with probiotics that can survive in the rumen has become a suitable alternative (Fon and Nsahlai 2013).

Therefore, this review summarizes the effects of direct-fed microbial (DFM) on rumen fermentation, methane inhibition, microbial populations and ruminant performance as growth, milk production and the efficiency of feed utilization.

2. Direct-fed microbial

The term "probiotic" is composed from two parts of Greek words: "pro" which means in favor and "biotic" which means life. The term probiotic has been defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller 1989). However, as pointed out by Vanbelle *et al.* (1990), many researchers accept that probiotic refers to "selected and concentrated viable counts of lactic acid bacteria *Lactobacillus*, *Streptococcus*". Moreover, Kmet *et al.* (1993) defined the term probiotics as "live cultures of microorganisms that are deliberately introduced into the rumen with the aim of improving animal health or nutrition". The Food and Drug Administration of USA has required feed manufacturers to use the term "direct-fed microbial" instead of probiotic (Miles and Bootwalla 1991) and has narrowed the definition to "a source of live, naturally occurring microorganisms" (Yoon and Stern 1995). Krehbiel *et al.* (2003) and Yang *et al.* (2004) defined the DFM as "alive, naturally occurring microorganisms that have been used to improve digestive function of livestock". The definition of DFM is very broad and may include specific and nonspecific yeast, fungi, bacteria, cell fragments, and filtrates (Sullivan and Martin 1999; Oetzel *et al.* 2007; Elghandour *et al*. 2014b). DFM grow in the rumen and beneficially modify its microbial ecosystem and/or fermentation characteristics. The intestinal tract may also provide a suitable habitat for DFM (Seo *et al.* 2010).

There are many different types of DFM being used in livestock production. They can be classified into three main categories; bacterial, fungal, and a combination of both. The bacterial DFM is the most common. The bacterial DFM strains may be classified as lactic acid producing bacteria (LAB), lactic acid utilizing bacteria (LUB), or other microorganisms. *Lactobacillus*, *Propionibacterium*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, and *Bacillus*, all of which are common microorganisms used in bacterial DFM for ruminants, in addition to other distinctive bacterial species such as *Megasphaera elsdenii* and *Prevotella bryantii* (Kung 2006; Seo *et al.* 2010). Development of this organism for ruminant animals should be continued with emphasis on optimizing dose and timing of administration. Success with such organisms could allow feedlot producers to decrease the time it takes to adapt cattle to a high concentrate diet. It could also be useful by reducing chronic acidosis in lactating cows (Kung 2006). The response to DFM was inconstant in ruminants; however, it has been positive in many experiments.

3. DfM mode of action

3.1. Bacterial DfM

The mode of action of DFM depends on many factors, such as dosages, feeding times and frequencies, and strains of DFM. Some of DFM act within the rumen while others impact the gastrointestinal tract (Puniya *et al*. 2015).

(1) Within rumen: The mode of action of different DFM sources within the rumen depends mainly on LAB and LUB. LAB might affect the rumen positively through preventing ruminal acidosis in dairy cows (Nocek *et al.* 2002) by facilitating the growth of ruminal microorganisms adapted to the presence of lactic acid in the rumen (Yoon and Stern 1995) and by stimulating LUB. LUB have been proposed as DFM that can decrease concentrations of lactate and maintain ruminal pH. *Megasphaera elsdenii* is the major lactate-utilizing bacterium in the rumen that prevents the drastic pH drops caused by accumulation of lactate in the rumen when fed a highly fermentable diet (Yang *et al.* 2004; Kung 2006) or prevents lactic acidosis in steers (Robinson *et al.* 1992). This bacteria simultaneously uses lactate, glucose, and maltose (Russell and Baldwin 1978) and would compete with lactate-producing organisms for substrate. During the feeding of readily degradable soluble carbohydrates, *M. elsdenii* seems to be the major ruminal lactate utilizer because *Selenomonas ruminantium* undergoes catabolite repression (Russell and Baldwin 1978) and is relatively acid-intolerant (Mackie and Gilchrist 1979).

Another bacterial species is the *Propionibacteria* which is naturally found in high numbers in the rumen of animals fed forage and medium concentrate diets (Kung 2006). Propionate is quantitatively the most important single precursor of glucose synthesis among volatile fatty acids (VFA), and tissue distribution of nutrient (Nagaraja *et al.* 1997). Certain

species of *Propionibacteria* were reported to modify rumen fermentation and increase the molar portion of ruminal propionate (Stein *et al.* 2006). It can ferment lactate to propionate in early lactation dairy cows (Reynolds *et al.* 2003; Kung 2006) resulting in increased hepatic glucose production (Stein *et al.* 2006), providing more substrates for lactose synthesis, improving energetic efficiency and reducing ketosis (Weiss *et al.* 2008). For growing ruminants and lactating cows, propionate has been estimated to account for 61 to 67% of glucose release (Reynolds *et al.* 1994; Huntington 2000). Also, increased propionate has been accompanied with a decrease in methane (CH_4) production according to the stoichiometric laws of chemical balance and its equation (van Soest 1994). When the acetate:propionate ratio decreases, CH_4 production declines, and energy retention by cattle would theoretically increase (Wolin 1960).

Feeding *Propionibacterium* increased protozoa especially *Entodinium* with decreased amylolytic bacteria in the rumen of feedlot steers (Ghorbani *et al.* 2002). The mechanism by which bacterial DFM stimulate protozoa remains unclear (Ghorbani *et al.* 2002).

(2) Within the post-ruminal gastrointestinal tract. Many proposals were adjusted to elucidate the mode of action of DFM within post-ruminal gastrointestinal tract (GIT) (Seo *et al.* 2010). DFM can inhibit or prevent pathogen like *Escherichia coli* establishment attached to the intestinal mucosa *via* hydrophobic interactions and limit pathogens from attaching to the enterocytic receptor or producing enterotoxins that can induce diarrhea (Lee *et al.* 2003; Kung 2006). LAB was able to adhere to the intestinal tract and protect animals against *Salmonella* (Frizzo *et al.* 2010). In addition to the role of LAB of producing lactate and acetate as main metabolic end-products, it had critical roles in penetrating microbial cells and interfering with essential cell function (Holzapfel *et al.* 1995).

Another mechanism is that DFM like LAB can produce antibacterial compounds such as bacteriocin and hydrogen peroxide that have a competitive exclusion and probiotic characteristics. Hydrogen peroxide can oxidize the sulfhydryl groups in metabolic enzymes such as glucose transport enzymes, hexokinase, and glycerol aldehyde-3-phosphate dehydrogenase causing glycolysis blocking (Carlsson *et al.* 1983; Dicks and Botes 2010). In contrast, LAB bacteriocins can inhibit the binding of substrates to the subunit of ribonucleotide reductase so as to interfer with DNA-synthesis of target microorganisms (Cotter *et al.* 2005; Dicks and Botes 2010).

A newly discovered mechanism is that DFM have the ability to modulate host immune function. In the GIT, various immune cells exist such as dendritic cells, natural killer cells, macrophages, neutrophils, and T and B lymphocytes that are aggregated in Peyer's patches, lamina propria, and intraepithelial regions (Krehbiel *et al.* 2003). After DFM are administered to the GIT, they are directly taken up by intestinal epithelial cells *via* transcytosis. Antigen presenting cells, macrophages or dendritic cells engulf them, finally stimulating an immune response (Dicks and Botes 2010). Various strains of LAB activate macrophages to produce cytokines that stimulate immune response. Matsuguchi *et al.* (2003) suggested that *Lactobacillus casei* Shirota and *Lactobacillus rhamnosus* Lr23 stimulated macrophages to secrete TNF-α or promote development of regulatory dendritic cells (Seo *et al.* 2010).

3.2. fungal DfM

Fungal DFM have been extensively used in ruminants for improving performance and normalizing rumen fermentation. *Saccharomyces cerevisiae* and *Aspergillus oryzae* are the most common used species (Elghandour *et al*. 2014a; Puniya *et al*. 2015).

A variety of mechanisms have been put to explain changes in ruminal fermentations and improvements in performance when ruminants are fed fungal-based DFM. The mode of action can be illustrated based on many facts. Yeast may have a buffering effect in the rumen by mediating the sharp drops in rumen pH (Elghandour *et al*. 2014a, b). Fungal cultures may improve the use of lactate by the ruminal organism, *Selenomonas ruminantium*, by providing a source of dicarboxcylic acids (e.g., malic acid) and other growth factors (Martin and Streeter 1995). Thus, yeast may help to buffer excess lactic acid production when ruminants are fed high concentrate diets (Kung 2006). Moreover, yeasts can remove oxygen on the surfaces of freshly ingested feed to maintain metabolic activity in the rumen (Newbold *et al.* 1996) and keep the rumen as anaerobic chamber. Another mechanism depends on the ability of yeast to decrease the redox potential in the rumen (Jouany *et al.* 1999) which provides a better condition for the growth of strict anaerobic cellulolytic bacteria, and stimulates their attachment to forage particles (Roger *et al.* 1990), and increases the initial rate of cellulolysis. In addition, *S. cerevisiae* was able to compete with other starch utilizing bacteria for fermentation of starch (Lynch and Martin 2002), which preventing lactate accumulation in the rumen, providing growth factors, such as organic acids or vitamins in the rumen, and resulting in stimulated ruminal cellulolytic bacteria and LUB (Chaucheyras *et al.* 1995).

The effects on buffering are subtle, as added yeast cannot prevent lactic acidosis if the rumen is challenged with a diet rich in fermentable carbohydrates (Dawson and Hopkins 1991; Aslan *et al.* 1995). The effect of fungal cultures on ruminal VFA has been inconsistent. Newbold *et al.* (1991) reported that fungal extracts had no effect or

tended to increase the rumen acetate: propionate ratio, while active yeast either had no effect or decreased the acetate: propionate ratio. There is no direct evidence that yeast or fungal extracts affect digestion or metabolism in the lower gut. However, the potential for such effects should not be overlooked.

4. Effect of DFM on ruminant performance

4.1. Preruminant calves

The young calves differ from the adult ruminants that they can digest a significant amount of ration nutrients in their intestine with the risk of intestinal proliferation of detrimental organisms that increased the chance for diarrhea and weight loss. Here come the roles of DFM administration which may obtain positively modified GIT function (Abu-Tarboush *et al.* 1996; Kung 2001). For dairy calves, repaid adaptation to solid feed by accelerating the establishment of ruminal and intestinal microorganisms and avoiding the establishment of enteropathogens, which often results in diarrhea, is the primary goal. In the experiment, Nakanishi *et al.* (1993) found that Holstein calves supplemented with yogurt containing *Lactobacillus acidophilus* tended to ruminate more at 30 d than untreated calves, indicating that *L*. *acidophilus* may promote ruminal development.

Dicks and Botes (2010) suggested that *Bifidobacteria* produces acetic and lactic acids at a ratio of 3:2, and that these acids may be more effective for the control of Gram-negative pathogens and yeasts in the GIT than *Lactobacillus* spp. because acetate is more effective against Gram-negative bacteria, moulds and yeasts (Gilliland 1989).

In other experiments, LAB was also inoculated into young calves to improve growth performance (Adams *et al.* 2008; Frizzo *et al.* 2010). Adams *et al.* (2008) examined the effect of *Propionibacterium jensenii* 702 (PJ702) on growth performance of young calves. There were improvements in weight gains with the treated group during both the pre-weaning and the weaning period with heavier calves final weight. Frizzo *et al.* (2010) fed young calves on milk replacer and a large quantity of spray-dried whey powder to generate an intestinal imbalance. Under these conditions, calves fed LAB had higher daily gain, total feed intake, and starter diet intake as well as lower fecal consistency index, indicating that diarrhea incidence was reduced.

The most common DFM species to young calves are *Lactobacillus* and *Streptococcus* species. Many reports have been documented a decreased incidence of diarrhea (Abu-Tarboush *et al.* 1996). Abu-Tarboush *et al.* (1996) found that feeding *L*. *acidophilus* 27SC to calves significantly lowered the incidence of diarrhea in calves. The decreased incidence of diarrhea might be associated with a consistently increased shedding of *Lactobacillus* (Gilliland *et al.* 1980; Jenny *et al.* 1991; Abu-Tarboush *et al.* 1996) and an inconsistent decreased shedding of coliforms (Bruce *et al.* 1979) in feces in response to supplements of *Lactobacillus*.

4.2. Dairy cows

Limited research has evaluated the efficiency of bacterial DFM for lactating dairy cows. High producing cows in early lactation would be the best candidates for such products because these cows are in negative energy balance and have diets that contain highly fermentable carbohydrates that sometimes lead to acidosis (Kung 2006). During the period of 3 wk prior to calving to 3 wk after calving (i.e., transition periods; Oetzel *et al.* 2007), cows may be subject to many metabolic disorders such as sub-acute acidosis as a result of calving stress, changing diets to rapidly fermented carbohydrate sources, and lactation (Oetzel *et al.* 2007; Chiquette *et al.* 2008). In this case, DFM should be used to improve performance of dairy cows through increasing dry matter intake, milk yield and milk protein content, higher blood glucose and insulin levels at the pre- and/or post-partum periods (Nocek *et al.* 2003; Nocek and Kautz 2006; Oetzel *et al.* 2007). In the study of Weiss *et al.* (2008), they supplemented dairy cows from 2 wk before anticipated calving to 119 d in milk with *Propionibacterium* P169. Cows fed P169 had lower concentrations of acetate with greater concentrations of propionate and butyrate. Plasma glucose and plasma β-hydroxybutyrate levels were not affected by DFM, with higher concentrations of plasma non-esterified fatty acids. Cows fed DFM produced similar amounts of milk with similar composition as cows fed the control diet. Calculated net energy used for milk production, maintenance, and body weight change were similar between treatments, but cows fed *Propionibacterium* P169 consumed less dry matter, which resulted in a 4.4% increase in energetic efficiency.

Chiquette *et al.* (2008) used *P. bryantii* 25A as a DFM to dairy cows in early lactation. They found that administration of *P*. *bryantii* 25A did not change milk yield, but tended to increase milk fat in accordance with increased acetate and butyrate concentrations in the rumen. *P*. *bryantii* 25A also decreased lactate concentration after 2–3 h of feeding compared with control treatments, thereby exhibiting the potential to prevent acidosis.

Exogenous cellulolytic bacteria have been studied as DFM to improve ruminal fermentation (Chiquette *et al.* 2008; Khattab *et al*. 2011). *Ruminococcus flavefaciens* NJ, was supplemented into the rumen of non-lactating dairy cows fed either a high concentrate or a high forage diet daily. *R*. *flavefaciens* NJ modified the abundance of other cellulolytic bacterial populations, and improved *in sacco* digestibility of timothy hay in the rumen when fed as part of a

high concentrate diet. The presence of *Aspergillus oryzae* or *S. cerevisiae*, or a change of concentrate to forage ratio in the diet did not succeed in establishing the new strain in the rumen.

4.3. Beef production

In finishing beef cattle, it is very important to prevent ruminal acidosis caused by highly fermentable feeds that are commonly be used. Beef cattle fed DFM showed improved growth performance, meat production, and feed efficiency in many experiments (Ghorbani *et al.* 2002; Krehbiel *et al.* 2003). DFM can have an important role on lowering newly received beef calves under stress on both newly received stressed calves and adult feedlot cattle. Newly received calves entering the feedlot heard undergo a variety of stress conditions, such as recent weaning, traction, and dehorning. Such conditions can alter microorganisms in the rumen and lower gut (Williams and Mahoney 1984), resulting in decreased performance and increased mortality and death loss. Administration of bacterial DFM to repopulate the gut might reduce these changes in the microbial population. The response to bacterial DFM might be greater when newly weaned and/or received calves are more prone to health problems. Krehbiel *et al.* (2003) administered 5×10^9 CFU LAB (*Enterococcus faecium*, *L. acidophilus*, *Bifidobacterium thermophilum*, and *Bifidobacterium longum*) to 466 newly received calves, to study the effects of LAB administration on health and performance. Daily gain did not differ among calves received DFM *vs*. those received no DFM. However, calves treated with DFM during their first antimicrobial treatment were less likely to be treated a second time within 96 h. In addition, the number of calves treated twice tended to be lower for calves administered DFM compared with calves received no DFM.

The effects of administrating DFM on stressed calves are limited. But in general, results suggest that the addition of DFM to the diet can improve health and performance of stressed stocker calves. These data suggested that DFM might improve recovery of morbid newly received feedlot calves.

Regarding to supplementing diets of feedlot with DFM, results showed that supplementing diets with LAB or LUB can improve feed efficiency and daily gain of feedlot cattle (Galyean *et al.* 2000). Huck *et al.* (1999) studied the effects of feeding *L. acidophilus* BG2FO4 and *Propionibacterium freudenreichii* P-63 as a DFM on growth performance and carcass characteristics of finishing heifers for 126 d. Feeding either *L. acidophilus* BG2FO4 or *P. freudenreichii* P-63 did not affect daily gain, dry matter intake (DMI), or feed efficiency. These authors suggested that growth performance of finishing cattle could be improved by targeting the appropriate DFM to a particular phase of production. Also, Krehbiel *et al.* (2003) summarized results of many reports and suggested that feeding bacterial DFM to feedlot cattle results in a 2.5 to 5% increase in daily gain and an approximately 2% improvement in feed efficiency, whereas DMI was inconsistent. In studies reviewed, carcass weight was generally increased by 6 to 7 kg.

Another role for DFM in case of feedlot cattle is reduction of *Escherichia coli* from GIT. The species of *E. coli* O157:H7 are commonly isolated from feedlot cattle. Feedlot cattle have been recognized as a host for *E. coli* O157:H7. This organism appears to be confined to the GIT and is shed in feces. Many studies suggested the possible application of bacterial DFM to reduce fecal shedding of *E. coli* O157:H7 from cattle. Based on those results, supplementing feed for cattle with certain DFM might decrease the incidence of *E. coli* O157:H7 in feedlot cattle. An increase in VFA, especially acetate, correlated with the reducing of *E. coli* O157:H7. For example, Ohya *et al.* (2000) used LAB of *Streptococcus bovis* LCB6 and *Lactobacillus gallinarum* LCB 12 to eliminate *E. coli* O157:H7 from experimentally infected Holstein calves.

5. Conclusion

It could be indicated that supplying DFM can contribute to the ability of the rumen ecosystem to manage lactic acid production and utilization can be beneficial, even for animals that do not have clinical acidosis.

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YEAST ADDITIVE AND ANIMAL PRODUCTION

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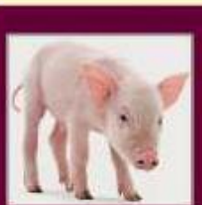
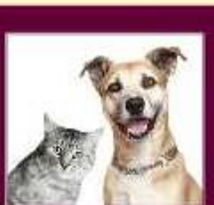
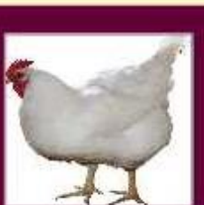
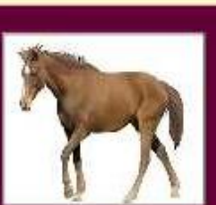

YEAST ADDITIVE AND ANIMAL PRODUCTION

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PREFACE

Addition of live yeasts *Saccharomyces cerevisiae* nowadays are increasingly applied as feed additives in ruminant's nutrition worldwide. Yeasts are considered as allochthonous microflora of the rumen environment and these can survive in the digestive tract for quite some time and interact there with the autochthonous microbial populations. The related positive benefits of yeast addition have been demonstrated on thedifferent rumen fermentation parameters. Some of theseparameters showtheir impacts on the growth and activity of fiber-degrading bacteria and fungi, the more stable rumen pH preventing lactate accumulation, the rumen microbial colonization, supply of growth factors to the rumenmicrobes, oxygen scavenging inducing more favorable conditions for the anaerobic microbial communities, and also on the set up of fermentative processes during the pre-weaning period; in addition to its role as an immunity stimulator. Modes of action of yeast probiotics depend on their viability and stability in the rumen ecosystem in addition to diets type.

In brief, the present book is quite a good collection of the knowledge available in the area of live microbial feed additives with special reference to the role of yeasts in ruminant nutrition. We are confident that this compilation of 7 chapters by virtue of its contents covering Chapter 1: History of yeast as feed additive; Chapter 2: Yeast: description and structure; Chapter 3: Mode of action of yeast in animal nutrition; Chapter 4: The rumen microbes and yeast culture in feed; Chapter 5: Yeast and the rumen fermentation activities and digestibility; Chapter 6: Yeast and milk production and fattening in ruminant; and Chapter 7: Yeast and non-ruminant animal performance; will popularize itself among the ruminant nutritionists, microbiologists, feed industrialists, students and researchers of related areas.

Editors also take this opportunity to sincerely thank all the contributors for their valuable efforts and also publisher for accepting this title of significance for publication.

This book is dedicated to ruminant nutritionists, microbiologists and feed industrialists.

Editors

CHAPTER 4

The rumen microbes and yeast culture in feed

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Abstract

The rumen is a unique ecosystem that contains millions of different microbes; vary between bacteria, protozoa, and fungi as the main microbial species. Its role is how to make the animal utilize poor quality feedstuffs through a chain of digestion processes. The role of yeast in this process is to make the rumen environment more favorable for both number and activity of the rumen microbes, which may positively improve the animal performance.

Key words: Feed, Fermentation, Microbes, Rumen, Yeast

INTRODUCTION

Live yeast has the ability to improve milk yield and weight gain of cattle as reported in literature. Yeast has the ability to stimulate bacterial activity within the rumen ecosystem. However, it remains unclear, if it induces a general stimulation of bacteria or if this stimulation is species specific (Pinloche *et al.*, 2013) (Fig. 7). However, many reports stated increased in cellulolytic microbial number and activity, when yeast was added to the feed diets (Kumar *et al.*, 2013). The ruminants rely on a symbiosis between the host and the rumen microbes, where the microbes supply protein, vitamins and organic acids for the animal host. Each of the absorbed energy, the protein digested in the abomasum, and glucose formation in the liver are all primarily derived from the microbial origins. In mature ruminants, little or none of the sugars and proteins initially present in the feed are directly incorporated into the animal; these are first processed via bacterial fermentation in the rumen (Dijkstra *et al.*, 2002). In fact, as much as 90% of the protein that reach the small intestine and up to 50% of the host energy requirements is provided by the microbes in the reticulum-rumen (Boyd *et al.*, 1991; Russell, 2002). The rumen contains one of the most complex, diverse and dense microbial ecologies known in the biological world (Choudhury *et al.*, 2015). The feed has a complex nature comprised of carbohydrate, fats, proteins, and minerals, and this could be the main possible

explanation for the microbial diversity in the rumen. In order to utilize feed components, microorganisms are either highly specialized to compete for a few category of the feeds or become widely adapted and are capable of using different type of nutrients. In the rumen, there are microbes that have the ability to alter their metabolism depending upon the availability of type of nutrients from the feed. In addition to the feed effects, another factor that selects for diversity is the ability of microorganisms to accomplish the most growth. Microbial growth is limited by both the quantity and quality of available feed. The feed is transformed into cells will dictate the survival. When the carbohydrate is converted to acetic, propionic or butyric acids, the production of carbon dioxide and methane are greater than other theoretical biochemical pathways. If the nature or amount of carbohydrate is changed, a heterolactic fermentation process will replace the acetic-butyric type by microbes that is more efficient for the growth of newer cells (Fellner, 2004). The rumen is colonized with protozoa, fungi, methanogenic Archaea, and bacterial populations. The latter is the most diverse group of microorganisms and represents more than half of the biomass, as its concentration is about10^{10} to 10^{11} cells/mL (Martin, 1994). By using bacteriological techniques, only about 200 bacterial species have been identified, isolated and their metabolism studied in pure culture, while new advances in rRNA based microbial ecology have revealed that many more bacterial species inhabit the rumen (Pitta *et al.*, 2010) are still uncultured.

The rumen fermentation process

The ruminants differ from non-ruminants (monogastric) in their method of feed digestion. The rumen is a large storage chamber in the foregut of numerous herbivores, where ingested food is first fermented by complex anaerobic microbial population. The rumen is the main site of the degradation and fermentation of different diet components (Fig. 8). These animals are able to digest high-fiber plant materials that are unsuitable for most non-ruminant animals. The rumen microorganism population consists of about 10^{10}bacteria, 10^5 ciliate protozoa, in addition to 10^3 phycomycetes fungi per mL (Rigobelo and Ávila, 2012), which ferment the diet to volatile fatty acids, microbial protein and vitamins. The establishment and maintenance of a stable microbial population is dependent upon the composition of diet, level of feeding, frequency of feeding and a number of microbial interactions (Kumar et. al, 2013a & b). The anaerobic microbial communities (i.e. bacteria, fungi, protozoa) make it very simple to digest and utilize the poor nutritive value feed components.

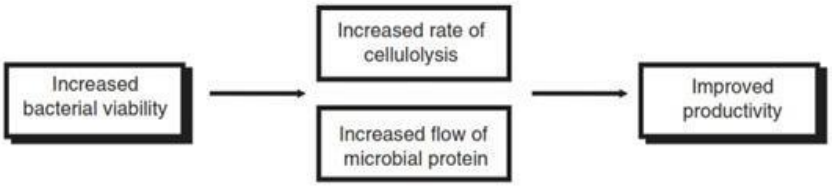


Fig. 7. Production response due to increased bacterial number (Beauchemine *et al.* (2006)

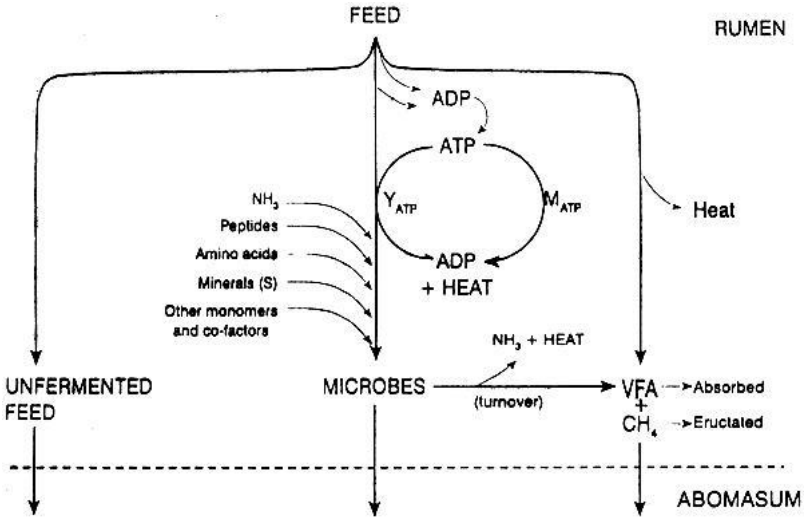


Fig. 8. Energetics of rumen fermentation (Leng, 1981)

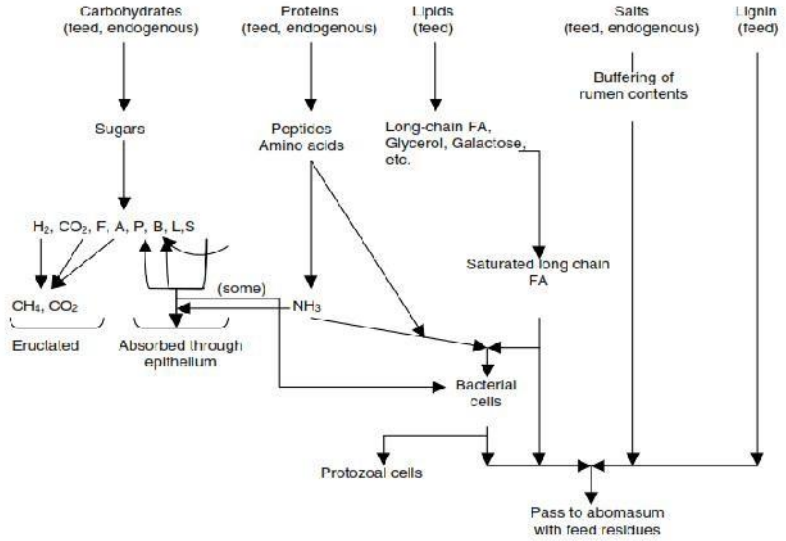


Fig. 9. The main microbial reactions in the rumen. FA: fatty acids, F:formic, A:acetic acid, B:butyric acid, L:lactic acid, S:succinic acid (Hobson, 1997)

The hydrolytic and fermentative processes in the rumen are due to the rumen microbes that provide the host animal with energetic and nitrogenous components, which are essential for the animal life (Fig. 9). The rumen contains a greater diversity of microflora of large numbers of bacteria, archaeons, ciliate protozoa, flagellate protozoa, anaerobic fungi and bacteriophage particles (Fig. 10).

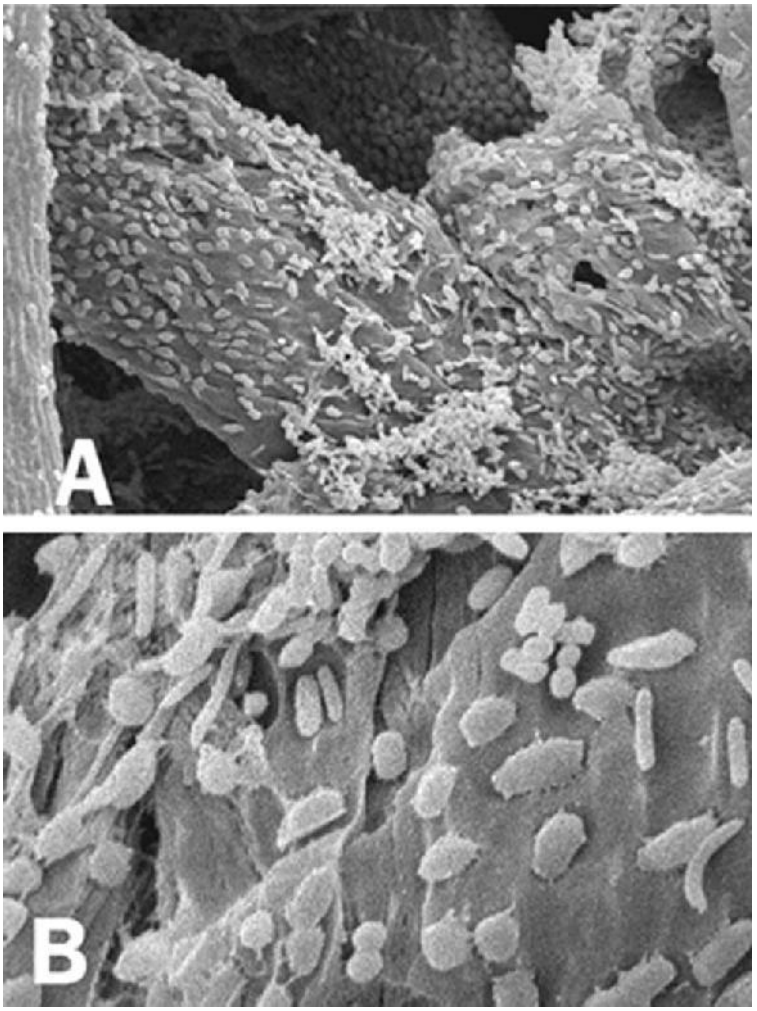


Fig. 10.Adherence of rumen bacteria to plant material (Miron *et al*., 2001)

Each gram of the rumen content contains about 10^{10} to 10^{11} cells (Stewart *et al*., 1997), where the fibrolytic bacteria is close to 10^{9} culturable cells/g of the rumen contents. The rumen bacteria can be divided into cellulolytic, amylolytic, hemicellulolytic, pectinolytic, proteolytic and other bacteria in the rumen (Table 4). In the ruminants, the main bacterial species are fiber-degrading species (i.e. *Ruminococcus albus*, *Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, and *Ruminococcus flavefaciens*). Another predominant species is *Prevotella ruminicola*,as these are able to utilize a broad range of substrates (i.e. sugars, proteins and starch) (Fonty *et al*., 1995). Quantitative PCR studies have indicatedthat the main cellulolytic species e.g. *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Ruminococcus albus* represent 1 to 5% of the total bacteria (Mosoni *et al*., 2007). *Streptococcus bovis*, *Selenomon as*

ruminantium, and *Megasphaera elsdeniiare* also present in high numbers, when higher levels of concentrate are fed to the animal (Mackie and Gilchrist, 1979).

Table 4. The main rumen bacterial species and their hydrolytic and fermentative capacity (Chaucheyras-Durand and Fonty, 2006).

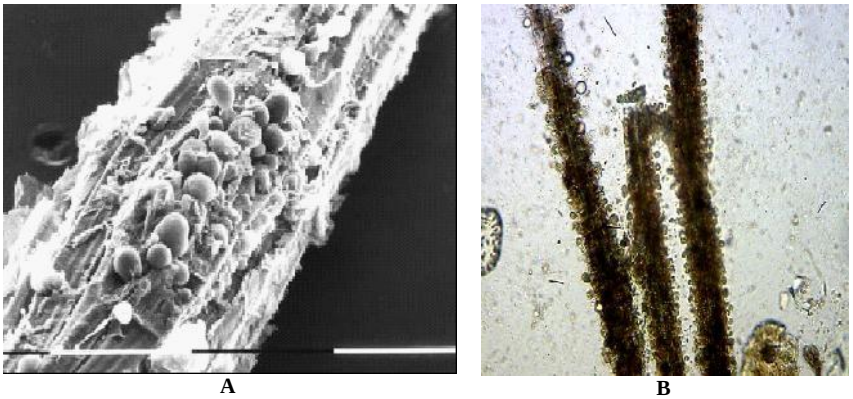
	cellulose	hemicelluloses	starch	pectines	proteins	triglycerides	cellobiose	maltose	glucose	glycerol	lactate	succinate	fumarate	peptides
Fibrobacter succinogenes	+	+	−	+	−	−	−	+	+	−				−
Ruminococcus albus	+	+	−	+	−		−	−	−	−				−
Ruminococcus flavefaciens	+	+	−	+	−		−	−	−	−				−
Butyrivibrio fibrisolvens	−	+	+	+	+		−	+	+					+
Prevotella ruminicola	−	+	+	+	+		−	+	+	−	−			+
Selenomonas ruminantium	−	−	+	+	+			+	+	−	+	+	−	+
Streptococcus bovis														
Ruminobacter amylophilus	−	−	+	−	+		−	+	−	−				−
Eubacterium ruminantium	−	−	−			−	−	+						
Megasphaera elsdenii	−	−	−	−	−	−		+	+	−	+		−	+
Anaerovibrio lipolytica	−	−	−	−	−	+	−	−	−	−				−
Methanobacterium ruminantium														
Wolinella succinogenes	−	−	−		−		−			−			+	−

Fibrobacter succinogenes is comparatively very active on crystalline cellulose and hemicelluloses (xylans) indicating its high fiber degrading potential. However, it is primarily able to use the products of cellulose hydrolysis (Kong *et al.*, 2012). On the other hand, *Ruminococcus albus* and *Ruminococcus flavefaciens* are active on cellulose, hemicellulose and pectins. Moreover, other bacterial species are considered as secondary fibrolytic species for example, *Prevotella ruminicola* and *Butyrivibrio fibrisolvens*, because these are not directly able to breakdown the cellulose. However, these bacterial species possess high carboxymethylcellulose-, pectin- and xylan-degrading activities and probably play a significant role in the overall fiber digestion in rumen (Suen *et al.*, 2011; Dodd *et al.*, 2011). Other species are also found in the rumen and occupy more specialized niches (i.e. *Anaerovibrio lipolytica*, *Veillonella alcalescens*, *Wolinella succinogenes*, etc). In general, methanogens are present in the rumen and are essential to ensure the proper functioning of the ecosystem; these use hydrogen and carbon dioxide to produce methane, which in turn is eructated by the animal into the environment (Kumar *et al.*, 2009; 2014). The archaeal or methanogens in the rumen is implicated in the removal of hydrogen via the synthesis and emission of methane thus, completing the anaerobic fermentation (Wolin *et al.*, 1997; Kumar *et al.*, 2009; 2014). Archaeal methanogens represent about $10^8$and 10^9 cells/ gthe rumen contents. The most common species of methanogens isolated from the rumen are strains of *Methanobrevibacter*, *Methanomicrobium* and *Methanobacterium* (Wolin *et al.*, 1997; Lumar et. al, 2012). Protozoa (Fig. 11) are mainly ciliates; their population is less abundant than the bacteria but owing to their great volume these can represent up to 50% of the total microbial biomass in the rumen. The ciliate protozoa (about 20 genera) play different roles within the ecosystem. These are able to degrade different substrates such as proteins, plant polymers and soluble

compounds, and engulf bacteria and fungi (Fig. 11), that contributes to regulate the microbial balance (Jouany *et al.*, 1995; Williams and Coleman, 1997; Chaucheyras-Durand *et al.*, 2012).

Fig. 11. ***Polyplastron multivesiculatum*** **protozoa engulfing fungal sporangia and bacteria (Williams *et al.*, 1994)**

Ciliate protozoa also participate in the fiber degradation (Chaucheyras-Durand *et al.*, 2012). These synthesize well-adapted enzymatic complex composed of cellulases and hemicellulases (Devillard *et al*., 2003; Béra-Maillet, *et al*., 2005). Each gram of the rumen content contains about 10^5 to 10^6 cells of protozoa. The most common species are Entodiniomorphs (i.e. *Entodinium*, *Epidinium*, *Eudiplodinium* and *Polyplastron*) and Holotrichs (i.e. *Isotricha*, *Dasytricha*), which use mainly the soluble sugars. Among protozoa, only Entodiniomorphs (*e.g.*, *Polyplastron*, *Epidinium,and Eudiplodinium*) are considered as cellulolytic. Different studies have reported that the removal of protozoa may negatively affect the fiber degradation in the rumen (Jouany *et al.*, 1988; Eugène *et al.*, 2004). Moreover, some anaerobic fungi (now 8 different genera), which are mainly implicated into cellulose breakdown are in the rumen (Fonty and Joblin, 1991; Gruninger *et al.*, 2014; Callaghan *et al.*, 2015; Dagar *et al.*, 2015). The rumen fungi are the sole anaerobic fungi that live attached to plant particles (Fig. 12). These exhibit strong cellulolytic and hemicellulolytic activities (Orpin and Joblin, 1997; Tripathi *et al.*, 2007a; Nagpal *et al.*, 2010). Anaerobic fungi are also involved in digestion of plant material (Dey *et al.*, 2004; Thareja *et al.*, 2006; Nagpal *et al.*, 2009a & 2011; Sirohi *et al.*, 2013a; Dagar *et al.*, 2014). Anaerobic fungi represent a homogenous phylogenetic group (phylum *Neocallimastigomycota*) and a very specialized functional group, as all species are fibrolytic (Orpin and Joblin, 1997; Gruninger *et al.*, 2014).



A **B**

Chaucheyras-Durand (2006) **Dagar (2011)**

Fig. 12 (A & B). The rumen fungi attached to a maize steam

The rumen fungi produce a very efficient set of cellulases and hemicellulases enzymes with higher specific activities than that of bacteria (Akin *et al.,* 1990). The fungi appear to be the first microorganisms to invade and commence digesting the structural plant components, beginning from the inside. The anaerobic rumen fungi reduce the tensile strength of these particles (Akin *et al.,* 1995) and thus, increase particle breakdown in rumination. These also possess esterase activities (contribute to the cleavage of ester bridges), which in turn link phenolic compounds of lignin to structural carbohydrates (Ljungdahl *et al.,* 2008; Qi *et al.,* 2011). The damage to digesta particles by fungi allows bacteria to colonize the plant cell materials. Therefore, these are thus extremely important initiators of fermentative breakdown of insoluble plant cell wall components, and their presence must reduce any lag time of fiber digestion in the rumen.

The number of anaerobic fungi depends on the type of diet fed to the animal. These might represent 5–10% of the total microbial biomass in the rumen despite of the fact that their number is much than bacteria. Due to their rhizoidal network the anaerobic fungi penetrate plant tissues and weaken the cell walls, which favor the accessibility of fiber degrading bacteria to their substrates (Fonty *et al.,* 1999). Eight different genera that have been identified up to now are *Neocallimastix, Orpinomyces*, *Piromyces*, *Caecomyces*, *Anaeromyces, Cyllamyces, Buwchfawromyces* and *Oontomyces*(Orpin and Joblin, 1997; Gruninger *et al.,* 2014; Callaghan *et al.,* 2015; Dagar *et al.,* 2015). Any improvement in the rumen hydrolytic and fermentative capacities will be reflected in more efficient ruminant production systems, as production level depends on the ability of the microbial ecosystem to convert organic matter into precursors of milk or meat (Chaucheyras-Durand and Fonty, 2006; Tripathi *et al.,* 2007b, Saxena *et al.,* 2010).

Effect of feeding yeast on the rumen microbes

Bacteria

An increase in the cellulolytic and lactic acid consuming bacteria (Pinloche *et al.,* 2013) in the rumen, appear to be the most consistent response to yeast

supplementation (Kumar *et al*., 2013; Mullins *et al.*, 2013). This effect depends on the yeast strain and dose. The increased bacterial count seems to be the central to the action of the yeast, driving both an increased rate of fiber digestion in the rumen and an increased rate of flow of microbial protein from the rumen (Offer, 1990; Martin and Nisbet, 1992; Wallace and Newbold, 1992; Dawson and Girad, 1997; Kung, 2001). What remains contentious is how small the amounts of yeast in the diet can stimulate microbial numbers in the rumen. The low numbers of non- *S. cerevisiae* yeast and molds occur naturally in the rumen (Lund, 1974), although growth of *S. cerevisiae* in the rumen seems unlikely (Chaucheyras-Durand *et al*., 1998). However, a lack of growth should not be confused with a lack of metabolic activity. They also found that *S. cerevisiae* was metabolically active in the rumen fluid for up to 30 hours. The yeast extract that did not contain whole cells did not stimulate bacterial growth in the same way the live *S. cerevisiae* did (Girard and Dawson, 1995). A number of mechanisms by which the yeast might stimulate bacterial numbers have been proposed (Rose, 1987; Wallace and Newbold, 1992). Nisbet and Martin (1990, 1993) that malate of *S. cerevisiae* stimulated the growth of the rumen bacterium *Selenomonas ruminantium* in medium containing lactic acid. In addition, Nisbet and Martin (1991) suggested that stimulation in the numbers of *Selenomonas ruminantium* by malate within the rumen yeast might occur *in vivo.* Girard *et al.* (1993) reported that *S. cerevisiae* increased the number of lactate-utilizing bacteria that could be recovered from the rumen fluid, with reductions in the rumen lactate levels in animals supplemented with *S. cerevisiae* (Williams *et al.*, 1991). An another mechanism can be illustrated based on the intracellular malic acid of *S. cerevisiae*, when incubated in autoclaved the rumen fluid and found no effect of adding malate to the rumen on the number of lactate-utilizing bacteria, however, malate did appear to stimulate the cellulolytic bacterial population and fiber digestion (Newbold *et al.*, 1996; Oeztuerk *et al.*, 2005).

Kung *et al.* (1982) and Martin and Streeter (1995) found that malate, at higher concentrations than that likely to be supplied by *S. cerevisiae*, stimulated the rumenfermentation. Martin and Nisbet (1992) suggested that yeast also might supply vitamins to the rumen. Both of niacin and thiamin are known to affect the rumen fermentation (Brent and Bartley, 1984). Chaucheyras *et al.* (1995) found that *in vitro*stimulation of the rumen fungi *Neocallimastix frontalis* by *S. cerevisiae*was at least partially due to thiamin in the yeast in addition to sufficient supply of vitamins to the *in vivo*stimulated fermentation. Moreover, Rose (1987) suggested that yeast might scavenge oxygen within the rumen, thus stimulating the growth of strict anaerobic bacteria in the rumen. Newbold *et al.* (1996) found a correlation between the ability of different yeast preparations to stimulate oxygen uptake by the rumen fluid and the ability of the yeast to stimulate the growth of the rumen bacteria. In general, the rumen is widely considered to be anaerobic; nevertheless, the rumengases, even in non fistulated animals, contains between 0.5to 1.0% oxygen (McArthur and Miltimore, 1962), and dissolved oxygen is detectable *in situ* (Hillman *et al.*, 1985). Many rumen microbes are highly sensitive to the presence ofoxygen (Loesche, 1969). The respiration-deficient mutants of *S. cerevisiae*, which were unable to remove oxygen from the rumen fluid, failed to stimulate bacterial numbers in the rumen-simulating fermenters, in conditions in which the parent strains, capable of scavenging oxygen, did stimulate the bacterial activity (Newbold *et al.*, 1996).

Different studies were carried out to test the effect of feeding yeast on the distribution of the rumen microbes. Mullins *et al.* (2013) analyzed the rumen microbial populations in lactating dairy cattle fed diets varying in carbohydrate profiles and *S. cerevisiae* fermented products. They stated no significant treatment effects on any target population.A significant interaction of treatment and dry matter intake was observed for *Eubacterium ruminantium*. An increased dry matter intake was associated with a quadratic decrease in *Eubacterium ruminantium* in control but with a quadratic increase in *E. ruminantium* populations in cows fed yeast fermented product. Kumar *et al.* (2013) studied the effect of daily feeding *S. cerevisiae*at the rate of 0.5 g/animal in the diet on the rumen microbial population in the buffalo bulls. The mean total of bacterial count in the yeast group was higherthan the control, which attributed to the positive effect of yeast to remove oxygen from the rumen. The comparable results were observed by Doleźal *et al.* (2005) and Kowalik *et al.* (2008). Pinloche *et al.* (2013) tested the ability of yeast to stimulate bacterial activity within the rumen using cannulated lactating cows, which received a daily ration (24 kg/day) of corn silage (61% of dry matter), concentrates (30% of dry matter), dehydrated alfalfa (9% of dry matter) and a minerals and vitamins mix (1% of dry matter).

The variation in the rumen bacterial community between treatments was assessed using Serial Analysis of V1 Ribosomal Sequence Tag (SARST-V1) and 454 pyrosequencing based on analysis of the 16S rRNA gene. The supplementation of probiotic yeast maintained a healthy fermentation in the rumen of lactating cattle (higher volatile fatty acids, higher rumen pH, and lower Eh and lactate) compared to the control diet. These improvements were accompanied with a shift in the main fibrolytic group (*Fibrobacter* and *Ruminococcus*) and lactate utilizing bacteria (*Megasphaera* and *Selenomonas*). The analysis of short V1 region of 16s rRNA gene (50–60bp) could give as much phylogenetic information as a longer read (454 pyrosequencing of 250bp). However, Mikulec *et al.* (2010) studied the influence of *S. cerevisiae* supplementation to the diet of fattening lambs on the growth performance and the rumen bacterial numbers. However, anaerobic and aerobic bacterial numbers were not significantly different. Among the isolated rumen bacteria, the most dominant was *Pediococcus* spp.in yeast supplemented animals.

Protozoa

Yeast supplementation has displayed positive impact on the growth and viability of the rumen microflora and the fermenting process in the rumen. However, the results have been inconsistent due to the conpounding effects of the ration composition, and variations in the strain of the yeast supplemented and the method of administration (Kumar *et al.*, 2013). Ghasemi *et al.* (2012) used four mature bulls fitted with the rumen cannulas to be fed the four experimental diets contained 0 or 5 g *S. cerevisiae* SC47(8×10^9cells/g) on the rumen digestion, fermentation and protozoa population. Yeast decreased the total counts of protozoa at 3 hours post feeding without modifying the counts of holotrich and entodiniomoroph. Moreover, Tripathi and Karim (2011) studied the effect of yeast cultures including *Kluyveromyces marximanus*, *S. cerevisiae*, and

Saccharomyces uvarum or their mix supplementation on the rumen ciliate protozoa population in growing lambs. The total numbers of ciliates, *Dasyticha*, *Entodinomorphs* and *Diplodinomorphs* were significantly different among lamb groups, but *Isotricha* numbers were nearly similar. The *Dasyticha* numbers were higher in *Kluyveromyces marximanus*, and *Entodinomorphs* and *Diplodinomorphs* numbers were higher in *S. uvarum* culture supplemented lambs. The population of ciliates was lowest in mixed yeast culture supplemented lambs. The population of ciliates was also lower in *S. cerevisiae* culture supplemented lambs, except that of *Entodinomorphs*. Kumar *et al.* (2013) studied the effect of *S. cerevisiae* CNCM I-1077 strain at the rate of 0.5 g/animal/day in the diet on the rumen microbial population in the buffalo bulls. The mean total protozoal count (10×10^4/mL of the rumen liquor) in the yeast culture supplemented group was higher than in the control group. Kowalik *et al.* (2011) stated that enrichment of the control diet with yeast metabolites increased the total protozoa population and the number of *Diplodinium* from $115\times10^4$to $146\times10^4$and from 2.5×10^4 to 6×10^4 / gdigesta, respectively. However, the number of representatives of the genus *Isotricha* decreased over 8 folds regardless of the additive used. The influences of *S. cerevisiae* on the number and genus of the rumen protozoa are inconsistent. Plata *et al.* (1994), and Al Ibrahim *et al.* (2010) observed increments in the total number of the rumen ciliates of cows fed yeast based preparations. However, Doreau and Jouany (1998) noted that addition of yeast preparation did not change the number of *Ophryoscolecidae* family represented by the genus *Entodinium*, *Epididinium* and *Diploplastron*. On the other hand, the family *Isotrichidae*, represented by the *Isotricha* spp., was in higher numbers, when the diet was supplemented with yeast.

In addition, Hristov *et al.* (2010) found that total protozoal, *Entodinium* spp., *Isotricha*, *Dasytricha*, *Epidinium*, *Ophryoscolex* and *Diplodinium* counts were not due to addition of *S. cerevisiae*. Lila *et al.* (2004) noted an unaffected protozoa population, whendifferent concentrations of a twin-strains of *S. cerevisiae*live cells were cultured on *in vitro* mixed rumen microorganisms fermentation of soluble potato starch, corn starch, and Sudangrass hay (60.5% on dry matter basis) plus concentrate mixture (39.5% on dry matter basis). Chaucheyras-Durand and Fonty (2002) investigated the effect of *S. cerevisiae*I-1077 (Levucell SC, containing 2×10^{10} cells/g), on microbial colonization of the rumen of newborn lambs received daily 0.2g yeast. They noted that pro t(**31** became established earlier in the lambs receiving yeast compared with co ı group of animals with a higher diversity of the protozoa community in presence of the yeast. They began to detect protozoa from the 16^{th} day after birth, compared to 21^{st}day after birth for control.

The similar results were also reported by Dobicki *et al.* (2006), who observed an increased number of ciliates in the rumen, when dried yeast was added to the cows' diet. The earlier colonization of the rumen by ciliates in yeast-lambs compared with controls indicates that the yeast favor earlier maturation of the microbial ecosystem, and that the climax of the rumen ecosystem is reached faster in presence of *S. cerevisiae*. Kowalik *et al.* (2012) evaluated the influence of live cells and metabolites of yeast *S. cerevisiae*(10g of live yeast or their metabolites were introduced into the rumen) in the diet on the numbers of ciliates in the rumen of three rumen-fistulated heifers fed a diet consisting of

88% meadow hay and 12% concentrate. They reportd that the total protozoa count did not differ significantly between the dietary supplements; however, diets supplemented with metabolites of yeast led to numerically higher ciliate numbers than those identified in animals fed the control diet without *S. cerevisiae.*Moreover, they showed that supplementation in the diet with yeast metabolites significantly increased concentration of genus *Entodinium* and *Ophryoscolex* compared to animals fed control diet or diet supplemented with live yeast cells. The concentration of protozoa from the genus *Diplodinium* were significantly lower in heifers fed live yeast than control or yeast metabolites supplemented diets. The effect of yeast preparation on the total protozoa was in accordance with the relationships described by Arakaki *et al.* (2000), who reported that total protozoa counts numerically increased, when metabolites were introduced into steers' diet. Arakaki *et al.* (2000) reported that number of *Diplodinium* in steers fed metabolites of yeast increased by 4%. Dobicki *et al.* (2006) have also found that metabolites of *S. cerevisiae*in diets increased the population of *Diplodinium* compared to control group of cows.

These results suggest that metabolites of *S. cerevisiae*contained soluble factors (*i.e.*, vitamins B, amino acids, organic acids, fumarate, malate, and aspartate) as well as cell membrane components (i.e. mannanes and β-glucanes) could stimulate growth of *Entodinium*. Brossard *et al.* (2006) suggest that number of protozoa from the family of *Ophryoscolecidae*, which represent approximately 90% of the total rumen protozoa, probably increased with the addition of *S. cerevisiae*. Galip (2006) evaluated the effect of *S. cerevisiae*, live yeast culture on protozoa count, and percent of different protozoa types using male Kivircik rams with the rumen cannula and received rations consisting of 70% grain diet and 30% alfalfa hay with daily addition of control group,5g or 10g of *S. cerevisiae*. The presence of *S. cerevisiae* had no significant effect on the protozoa absolute numbers. However, after *S. cerevisiae* supplementation, increased percent of *Diplodinium*, *Dasytrichia* were observed. Addition of *S. cerevisiae* decreased the percent of *Epidinium.*

Fungi

Few studies to assess the effect of yeast addition on the rumen fungi have been reported. This may be due to the difficult isolation and characterization of the rumen fungi (Nagpal et. al, 2009b). However, recently described mole **32** techniques made it possible to resolve the identity of these rumen fungi up to the species level (Dagar *et al.*, 2011; Sirohi *et al.*,, 2013b). Mao *et al.* (2013) investigated the effects of a *S. cerevisiae* fermentation product on *in vitro*rumen fermentation of single forage and mixed diets, and found that the fungal population was greater with *S. cerevisiae* addition compared to control.

Conclusions

The effects of adding yeast to the diets of animals will affect different group of microbes in the rumen. The effects may vary among different rumen microbial species. But in general an improved activity of the rumenand animal productivity can be observed after *S. cerevisiae* supplementation in animal's diet.

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CHAPTER 7

Yeast and non- ruminant animal performance

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Abstract

The genus *Saccharomyces* has many different species with hundreds of strains; a few of them are used as feed additives for ruminants and non-ruminants nutrition. In case of non-ruminants, addition of yeast in the diet has many beneficial effects including; reducing bacterial toxins in the gastrointestinal tract, adherence of flagellate bacteria, and reinforcement of mucosal integrity and intestinal cells. All previous benefits will aid in optimizing the growth potential of the monogastric animal.

Key words: Non-ruminant, Performance, Yeast

INTRODUCTION

In monogastric (*i.e.*, non-ruminants), the gastrointestinal tract and digestion processes are completely different than those of ruminant animals. The digestive tract in each animal species will be different than the other. But in general, the gastrointestinal tract is composing of different parts including stomach, small intestine (i.e. duodenum, jejunum and ileum) and large intestine (i.e. caecum, colon and rectum). In the stomach compartment, the ingested feed is strongly acidified by the acid excreted by the mucosa, and pepsin to start protein digestion. In the small intestine, two important steps occur for the digested feeds. The first step is the secretion of various digestive enzymes to separate the different nutrients into absorbable constituents that can be absorbed in the second step. The mucous surface starts to absorb the separate nutrients constituents. The gastrointestinal tract possesses a very large mucous surface that arises mainly from the fingershaped protrusions villi of the small intestine wall. The absorbed nutrients pass into the blood stream. Non-absorbed constituents of the diet reach the large intestine, where these are broken down and digested, mainly by the intestinal microflora. In case of horse, the digestive system and digestion process is not as in the other monogastric animals. Horses have unique digestive system that able them to utilize fiber in their diet. The primary site of

non-structural carbohydrate digestion (*i.e.*, the foregut) is dramatically smaller, when compared to the site for structural carbohydrate degradation (*i.e*., hindgut) of the mature horse (Argenzio *et al*., 1974; Morgan, 2006). The hindgut contains microbes making horse efficiently digest fiber, roughage and make them the main component of the mature horse's diet (Morgan, 2006, Salem *et al*., 2015). Therefore, it is expected that addition of yeast to the diet of non-ruminants will acts in different way with different species.

Pigs

The yeast direct-fed microbials are widely used in pig nutrition. It could play an important role in the transition of nursing pigs from milk to a solid diet. The yeast administration has been demonstrated to be useful in improving the nitrogen metabolism presumably by enhancing the fihe ogen metabolism presumably by enhancing The yeast has the ability to stimulate digestion and aid in maintaining the microbial equilibrium in the gut of young pigs. In addition, yeast possesses enzymes, vitamins, and other nutrients or growth factors have been proposed to produce beneficial production responses in pigs (Kornegay *et al*., 1995). The yeast can directly affect the microflora by yeast cell wall components or directly by reducing the pathogenic bacteria and toxic metabolites and subsequently improve the animal health and growth performance (Anderson *et al*., 1999). The live yeast supplementation may improve the disease resistance and performance through stimulation of the immune system and maintenance of a beneficial intestinal environment(van Heugten *et al*., 2003; Collier *et al*., 2011). Using immunomodulators to modulate the immune function of animals is considered a potential means to improve their performance and health status (Li *et al*., 2007).

Adding the a *S. cerevisiae* fermentation product in the gestation and lactation diets has the potential to improve litter body weight gain during lactation by improving the maternal protein utilization or improve the maternal health status, or increase milk production without affecting the nutrient composition of the colostrum and milk (Shen *et al*., 2011). Van Heugten *et al.* (2003) conducted two experiments to evaluate the effects of live yeast of *S. cerevisiae* SC47 supplementation on nursery pig performance, fecal microflora and nutrient digestibility to determine, whether live yeast could replace antibiotics and growth-promoting concentrations of Zn and Cu in nursery pigs. They found that yeast supplementation did not affect the growth performance of pigs. However, yeast supplementation decreased fecal total bacteria and lactobacilli. The dietary yeast resulted in a greater yeast count in feces of pigs. The yeast decreased the digestibility of dry matter, fat, and gross energy in the prestarter and starter phases. Shen *et al.* (2011) studied the effects of adding a *S. cerevisiae*fermentation product (i.e. yeast cell wall fragments, residual yeast cells, and the media used during fermentation) to the gestation and lactation diets on the performance of sows and their progeny. The sows fed a diet with 12.0 and 15.0g of fermentation product/day. The sows fed the yeast fermentation product tended to have increased total litter weaning weight and litter body weight gain. The neutrophil count was decreased by adding the fermentation product on day 110 of gestation and day 17 of lactation, whereas a decreased white blood cell count was observed only on day 110 of gestation. In addition, total tract apparent

nutrient digestibility values of ash, crude protein, dry matter, and ether extract. The colostrum and milk content from protein, IgG did not differ between the treatments. A salmonella infection trial in pigs evaluated the addition of anaerobically fermented yeast products (*S. cerevisiae*) to the starter diet in weaned pigs following a challenge with 10^9 cells of *Salmonella Typhimurium* DT104. The pigs receiving the yeast supplement showed slightly better body weight gains post-infection than non-supplemented controls but the supplemented group had a tendency towards increased salmonella shedding (Price *et al*., 2010).

Poultry

There have been debates about the use of antibiotics as growth promoters in animal nutrition, due to the probable relationship with resistance to antibiotics used in human medicine, or the presence of antibiotic residues in products of animal origin intended for human consumption. Hence, it was necessary to search for an alternative products that could replace the antibiotics used as growth promoters without causing negative effects on the productivity or product quality. Thereby, it is very important to use safer alternative, which are products made from living microbes (Otutumi *et al*., 2012). In poultry, several studies have been made and continue being developed with the use of probiotics and *S. cerevisiae*. The inconsistent results have been a constraint for the promotion of their use. Gheisari and Kholeghipour (2008) included four levels (0, 0.1, 0.2 and 0.3%) of two forms (i.e. powdery and granular) of live yeast of *S. cerevisiae*to assess the effect of yeast on the performance, humoral immune responses titers against influenza disease virus and blood parameters in three dayold commercial male broiler chicks. The results showed that the use of live yeast is not affected body weight, daily gain, feed intake, and feed conversion. However, treatment containing 0.3% yeast (i.e. powder) in comparison to granular and control groups had a higher body weight, daily gainand feed intake. The use of yeast had no significant effect on antibody titers against influenza disease virus, but at 38^{th}day of age chicks fed 0.2% powdery yeast had a higher antibody titers against influenza disease virus than the control group ($P<0.05$). The chickens fed diets containing granular yeast had a lower heterophil to lymphocyte ratio in compare to the control chickens. The diets containing 0.1 and 0.2% powdery yeast lowered serum cholesterol and increased serum highdensity lipoprotein concentrations of the chickens, respectively. The action mechanism of live yeast for improving performance is not fully understood,however, there are two probabilistic explanations:(i) action of yeast is most probably supporting the growth of lactic acid bacteria,(ii) a competitive exclusion of pathogenic bacteria by yeast and its products, especially the cell wall component (Onifade, 1998). The yeast cell wall is containing mannan, glucan and chitinthat have been known as immune-stimulant (Li and Gatlin, 2003; Rodriguez *et al*., 2003). Four novel applications of yeast in animal production have emerged, which are outside the conventional uses; including: (l) yeast being used specifically for one of its metabolic products (*e.g.* the use of *Phaffia rhodozyma* carotenoids for egg-yolk color); (2) the ability of yeast to influence the normal microbial population within caecum; (3) the role of some yeasts as a modifier of the livestock gut microflora and stimulator of immune system; (4) the use of *S. cerevisiae*, when added to feed, to counteract

aflatoxicosis in broiler chickens and ducklings. The models describing the effects of yeast on animal production are currently based on the ability of yeast strains to stimulate the growth and activities of gastrointestinal bacteria, however, the stimulatory characteristics may not be common to all the strains of yeast.

The ability of *S. cerevisiae* to the amelioration of aflatoxicosis in Japanese quails was examined (Parlat *et al*., 2001). They incorporated yeast into the diet at 1g/kg to reduce the deleterious effects of 2.5mg total aflatoxins. The presence of aflatoxins in the diet significantly and dramatically decreased the food consumption and body-weight gain from the first week onward. The addition of yeast to the aflatoxins containing diet significantly reduced these deleterious effects on feed consumption, bodyweight gain and feed conversion ratio. *S. cerevisiae* and its cell wall component would minimize the adverse effects of aflatoxins in poultry on the basis of biological degradation (Raju and Devegowda, 2000). The additional benefits of *S. cerevisiae* may be due to the stimulation of the immune response (Savage *et al*., 1996), alteration of intestinal microbial environment (Newman, 1994) and producing enzymes for gut microflora to enhance the nutrients bioavailability (Raju and Devegowda, 2000).

The role of *S. cerevisiae* on aflatoxins detoxification may be attributed to its ability to produce the biological enzymes that interact with the aflatoxins molecules (Stanley *et al*., 1993) and to other growth promoting effects (Raju and Devegowda, 2000). It was also reported that *S. cerevisiae* has been known to alter the stress in animals by providing a source of vitamins, enzymes and growth protein for reducing stress, to enhance the biological value of nitrogen compounds along the digestive tract (Stanley *et al*., 1993). Gao *et al.* (2008) evaluated the effect of supplemental yeast culture (Diamond V XP) at 0, 2.5, 5.0, and 7.5g/kg of yeast on one day old Arbor Acres chicks performance, digestion, mucosal development, and immunomodulatory functions for 42 days. The yeast supplementation at 2.5g/kg improved the average daily gain and feed conversion during grower and overall periods. The yeast culture supplementation increased the digestibility of calcium and phosphorus on day 35, but did not affect the protein retention and energy digestibility. The yeast supplementation increased villus height to crypt depth ratios in the duodenum and jejunum (day 42) and ileum (day 21) in broilers fed 2.5g/kg of yeast was observed. The yeast culture increased antibody titers to Newcastle disease virus, serum lysozyme activity, and IgM and secretary IgA concentrations in the duodenum.

Rabbits

In monogastric animals such as the rabbit, probiotics would affect mainly the hindgut microbiota (i.e. caecum and proximal colon) with their very diverse and active bacterial community (Carabaño *et al*., 2006; Fortun-Lamothe and Boullier, 2007). However, live microbes would be beneficial, only if they survive to the environmental conditions, such as the pelleting processes or the transit through the gastrointestinal tract (Falcão-e-Cunha *et al*., 2007). The live yeast supplementation would affects mainly on the digestive health in the young rabbit, especially under non-optimal breeding conditions (Maertens and De Groote, 1992). But their mechanism of action remained to be elucidated, since, very few dealt specifically with the survival of yeast in the caecum and on their

potential impact on the microbiota activity. Kimsé *et al.* (2012) found that when live yeast *S. cerevisiae* NCYC Sc 47 was added at 1 or 10g/kg of basal diet to the diet of rabbits (3 days old) did not modify the digestibility coefficients of dry matter, organic matter and crude protein. Each of weight gain, feed intake and feed efficiency were not affected by live yeast addition. During the first week of the experiment (35–42 days of age) no mortality or morbidity were registered.

Horses

The digestive ecosystem of the horse is populated with bacteria, protozoa and fungi, each with a specific role and function in the breakdown of forage. The most frequently occurring bacteria are made up of three groups: amylolytic bacteria, representing 42% of the total flora, have a role in breaking down starch; fibrolytic bacteria, comprising 28% of the total flora, have a role inbreaking down of the cell wall; and lactic acid utilizing bacteria (14%) of total flora. There are common practices during horse nutrition to supplement the roughage diets with starch-based concentrates to meet the nutrient requirements (NRC, 1989). When, non-degraded starch escapes small intestinal digestion and reaches the hindgut, some changes including the altered microbial populations, decreased pH, increased propionate concentration, and decreased fiber digestion (Medina *et al*., 2002), and also cause an increased risk of acidosis, colic, or laminitis in the horse (Bailey *et al*., 2003).

The contribution of animal probiotics, notably *S. cerevisiae*, has also been the subject of research across the world. Medina *et al.* (2002) used crossbred male mature horses fed daily at the same level of intake either a high fiber or a highstarch diet without or with 10g of a*S. cerevisiae* preparation. Supplementing the *S. cerevisiae* preparation increased the concentration of viable yeast cells (averaging 4.3×10^6 and 4.5×10^4 cells/mL) in the cecal and colonic contents, respectively. However, yeast administration had almost no effect on microbial counts in the cecum and colon. *S. cerevisiae* appeared to modify pH, concentrations of lactic acid and ammonia-N, acetate and butyrate concentration with high starch. The effects of the *S. cerevisiae* preparations were greater in the cecum than in the colon coinciding with the abundance of yeast cells.

The effect of the addition of a *S. cerevisiae* appeared to limit the extent of undesirable changes in the intestinal ecosystem of the horse, when the digestion of starch in the small intestine was saturated. Jouany *et al.* (2008) used eight crossbred male horses in pairs to be fed a highfi2008) used starch diets with or without *S. cerevisiae* supplementation. The supplementation with yeast improved acid detergent fiber digestibility and stimulated dry matter and neutral detergent fiber intakes, but had no effect on the mean retention time of the feed particles of solid digesta. Elghandour *et al*. (2014) evaluated the effect of inclusion of *S. cerevisiae* containing 1×10^{10}/g at 0, 1.25, 2.5 and 5mg/g dry matter on *in vitro* fermentative capacity of nine low quality fibrous forages. Addition of *S. cerevisiae* at levels of 2.5 to 5.0g/kg dry matter improved fecal fermentation capacity of those low quality forages. Moreover, Salem et al. (2015) observed improved feed intake and nutrients digestibility when fed yeast culture to mares.

Dogs and cats

The low number of studies investigated the effect of addition of *S. cerevisiae* to dogs and cats feeds. Robertson *et al.* (1976) used Beagle bitches to be fed dried brewers grains supplemented with 5% dried brewers yeast for 12 months. No significant differences in feed consumption, body weight between treatments at the end of 12 months of feeding between treated and control groups were detected. Middelbos *et al.* (2007) evaluated the addition of the yeast cell wall preparationas a dietary supplement for adult dogs and reported thatdry matter intake was not affected by the supplementation. Similarly, individual nutrient intakes (i.e. organic matter, crude protein, acid hydrolyzed fat, total dietary fiber concentrations, insoluble dietary fiber concentrations, and soluble dietary fiber concentrations) were not affected by the supplementation of yeast cell wall preparation.

Ileal digestibility of dry matter, organic matter, crude protein, fat, and gross energy tended to respond cubically to yeast cell wall preparation supplementation, with the greatest values at the 0.25% level. In contrast to ileal digestibility values, the 0.25% yeast cell wall preparation supplementation level had lower total tract digestibilities,whereas total white blood cell count tended to decrease quadratically with yeast cell wall preparation supplementation. However, neutrophil and lymphocyte counts were not affected; but, monocyte concentrations, decreased linearly, whereas eosinophil concentrations tended to decrease with increasing yeast cell wall preparation supplementation. Of the serum immunoglobulins, IgG and IgM concentrations were not affected, whereas ileal IgA concentrations tended to increase quadratically with yeast cell wall preparation supplementation, with the greatest value at the 0.25% yeast cell wall.

Conclusions

As was noted improved performance in ruminant animals, the monogastric animals showed the improved performance. The mode of action in monogastric animals completely differ than that of ruminants. The improved immunity also was observed on yeast supplementation.

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III.- JUSTIFICACIÓN

Debido al incremento en costos de producción por alimentación en animales y a la competencia que existe con la alimentación humana, principalmente con granos, es necesaria la búsqueda de alternativas alimenticias, como lo es el uso de forrajes de baja calidad, sin embargo, estos no aportan los nutrientes suficientes, por lo que la levadura *Saccharomyces cerevisiae* puede ser una opción viable para mejorar la digestibilidad de los mismos y así incrementar el valor nutritivo que pueden aportar los forrajes de baja calidad a los animales.

IV.- HIPÓTESIS

La inclusión de *Saccharomyces cerevisiae* mejorará el valor nutricional y algunos patrones de fermentación cecal o ruminal de los forrajes de baja calidad para la nutrición de los animales.

V.- OBJETIVOS

5.1.- Objetivo General

Evaluar el impacto de *Saccharomyces cerevisiae* como un probiótico sobre el valor nutritivo de algunos forrajes de baja calidad para los animales.

5.2.- Objetivos Específicos

- Evaluar el impacto de *Saccharomyces cerevisiae* CNCM I-1077 (LEVUCELL) como un probiótico sobre el valor nutritivo de algunos forrajes de baja calidad en rumiantes.
- Evaluar el impacto de diferentes niveles de *Saccharomyces cerevisiae (*procreatin7) como un probiótico sobre el valor nutritivo de algunos forrajes de baja calidad en caballos.
- Revisar artículos publicados recientemente sobre el uso de los microbios como aditivos sobre el valor nutritivo de los forrajes de baja calidad.
- Evaluar el impacto de diferentes niveles de tres productos comerciales de *Saccharomyces cerevisiae. (*procreatin 7®, Biosaf SC47®, Fermipan F53®) como un probiótico sobre el valor nutritivo de dos dietas balanceadas con alto y bajo contenido de proteína.

VI.- MATERIALES Y MÉTODOS

ARTÍCULO 1.

Titulo:

In vitro fermentative capacity of equine fecal inocula of nine fibrous forages in presence of different doses of *Saccharomyces cerevisiae*

Revista:

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Tres muestras individuales de cada uno de los alimentos fibrosos de rastrojo de maíz y mazorca (*Zea mays*), paja de avena (*Avena sativa*), bagazo de caña de azúcar y hojas (*Saccharum officinarum*), hojas de pasto llanero (*Andropogon gayanus*), hojas de pasto Taiwán (*Pennisetum purpureum*), paja de sorgo (*Sorghum vulgare*) y hojas de pasto estrella (*Cyinodon plectostachyus*) fueron recolectadas por triplicado al azar y de forma manual en diferentes sitios del Estado de México en México. Las muestras de rastrojo de maíz, paja y hojas se colectaron en la última etapa de madurez y se secaron a 60 °C durante 48 h en una estufa de aire forzado hasta alcanzar peso constante, se molieron en un molino Wiley para pasar un tamiz de 1 mm y se almacenaron en bolsas de plástico para su posterior análisis químico y su producción de gas (GP) *in vitro*. Un aditivo para piensos de *Saccharomyces cerevisiae* disponible comercialmente (Procreatin 7®, Safmex / Fermex SA de CV, Toluca, México) en forma de polvo conteniendo 1×10^{10} UFC / g, se utilizó en cuatro niveles (/ g de MS de sustrato) de: control (0 mg), baja (1.25 mg), media (2.50 mg) y alta (5.00 mg). Las Muestras de alimento se incubaron con las dosis de levadura que se agregaron a las botellas inmediatamente antes de la incubación. Antes de iniciar los tratamientos se preparó una cantidad suficiente de solución de levadura en agua destilada a fin de obtener la dosis suficiente del cultivo en 1 ml de solución.

Incubaciones *in vitro*

El Inóculo fecal se colectó de cuatro caballos adultos que van de 5 a 8 años de edad y un peso de 480 ± 20.1 kg. Los caballos fueron alimentados diariamente en una

cantidad de concentrado comercial (PURINA®, Toluca, México) y heno de avena *ad libitum*. Permanentemente los caballos tuvieron agua fresca disponible.

La técnica de GP empleada fue la descrita por Theodorou et al. [18]. El contenido fecal se recolectó directamente del recto de cada caballo e inmediatamente se trasladó al laboratorio para la incubación *in vitro*. El contenido fecal se combinó con el medio de cultivo en una proporción de 1: 4 y se mantuvieron gaseando con CO2 inmediatamente después de la extracción y durante el proceso de incubación. El inóculo fecal mezclado con el medio de cultivo se utilizó para inocular tres series idénticas (corridas) de botellas que contienen 1 g MS de cada uno de los sustratos de los alimentos fibrosos. Para cada inóculo, también se incluyeron tres controles sin sustratos (blancos). Esto dio lugar a un número total de 324 botellas para la GP (9 alimentos fibrosos × 3 muestras individuales × 3 corridas × 4 dosis de levadura). Una vez que se llenaron todas las botellas, se cerraron inmediatamente con tapones de goma, se agitaron y se colocaron en la incubadora a 39 ºC. Las lecturas de la producción de gas se realizaron a las 2, 4, 6, 8, 10, 12, 24 y 48 h después de la inoculación mediante la técnica del lector de presión (Extech instruments, Waltham, CT, EE.UU.). Al final de la incubación (es decir, 72 h), las botellas fueron destapadas y el pH se midió utilizando un medidor de pH (Conductronic pH15, Puebla, México)

Cálculos y análisis estadístico

Para estimar los parámetros cinéticos de la PG, los resultados (ml / g MS) se ajustaron utilizando la opción NLIN de SAS [19] de acuerdo a France et al. [20] como sigue:

$A = b \times (1 - e^{-c\,(t-L)})$

Dónde: A es el volumen de PG en el tiempo t; b es la asintótica de PG (ml / g MS); c es la velocidad de PG (/ h), y L (h) es el lapso de tiempo antes de la PG.

El diseño experimental fue un diseño completamente al azar teniendo en cuenta, como factores fijos, las especies de alimento (S) y las dosis de cultivo de levadura (D) en el modelo lineal [21]. Los datos de cada una de las tres corridas con la misma

muestra se promediaron antes del análisis estadístico. Los valores medios de cada muestra individual dentro de cada especie (es decir, tres muestras de cada una) fueron usadas como la unidad experimental. El modelo estadístico fue:

$Yijk = \mu + S_j + D_k + (S \times D)_{jk} + E_{ijklm}$

dónde: Y_{ijk} = es cada observación de la iésima especie fibrosa (S_i) cuando se incuba en la j-ésima levadura (D_j; dosis de cultivo de levadura); μ es la media general; S_i (i = 1-9) es el efecto de los alimentos; C_j es el efecto de la dosis de levadura (j = 1-4); $(S \times D)_{jk}$ es la interacción entre los alimentos y la dosis de levadura y E_{ijklm} es el error experimental. Contrastes de polinomios lineares y cuadráticos se utilizaron para examinar las respuestas de los alimentos al incrementar los niveles de adición de la levadura.

ARTICULO 2.

Titulo:

Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on *in vitro* gas production kinetics and degradability of four fibrous feeds

Revista:

Italian Journal of Animal Science 13 (2014), 295-301

Tres muestras individuales de cada uno de llos alimentos fibrosos: rastrojo de maíz, paja de avena, bagazo de caña de azúcar y paja de sorgo se recolectaron al azar y de forma manual por triplicado de diferentes sitios en el Estado de Estado de México. Las muestras se secaron a 60ºC durante 48 h en una estufa de aire forzado hasta peso constante, se molieron en un molino Wiley para pasar un tamiz de 1 mm y se almacenaron en bolsas de plástico para la posterior determinación de su composición química y la producción de gas *in vitro*. Cuatro niveles de un producto comercial de levadura (*Saccharomyces cerevisiae* I-1077, Levucell® SC20, LALLEAND Animal Nutrition SA, Blagmaccedex, Francia) conteniendo 1×10^{10} levaduras por gramo. Las dosis de levadura fueron (/ g DM): control (0 mg,), baja (4 mg), media (8 mg) y alta (12 mg). Las muestras de alimento se incubaron con dosis de levadura que se agregaron en las botellas inmediatamente antes de la incubación (método directo) o

se pre-incubaron por 72 h a temperatura ambiente. Antes de iniciar los tratamientos se preparó una cantidad suficiente de solución de levadura en agua destilada a fin de obtener la dosis suficiente del cultivo en 1 ml de solución.

Incubaciones *in vitro*

El inóculo ruminal se obtuvo de dos vacas suizas (400 a 450 kg de peso corporal), con cánula ruminal permanente. Las vacas fueron alimentadas *ad libitum* con una ración total compuesta de 50:50 de forraje de heno de alfalfa y de un concentrado comercial (PURINA®, Toluca, México) que contenía (g/kg) 147.3 CP, 160.4 NDF, y 277 ADF; formulado para satisfacer todas sus necesidades de nutrientes (NRC, 2001). Agua fresca estaba disponible a las vacas en todo momento durante la fase de recogida de inóculo ruminal.

El contenido ruminal de cada vaca se obtuvo antes de la comida de la mañana, se gaseo con CO2 se mezcló y se filtró a través de cuatro capas de gasa en un matraz con espacio superior libre de O_2. Las muestras (1 g) de cada alimento se pesaron en frascos de suero de 120 ml con la adición apropiada de *S. cerevisiae,* dosis / g MS. Consecutivamente, se añadieron 10 ml de fluido ruminal libre de partículas, 40 ml de la solución tampón de acuerdo con Goering y Van Soest (1970), sin añadir tripticasa quedando en una proporción 1: 4(v / v) .

Durante las incubaciones, se utilizaron 4 piensos con 3 muestras individuales de cada uno, con las 4 dosis de *S. cerevisiae* en 2 métodos de aplicación (adición directa o 72 h previas tratamientos) de *S. cerevisiae* y 4 botellas (repeticiones) se utilizaron para cada muestra incubada durante 3 corridas de incubación. Una vez que se llenaron todas las botellas, se cerraron inmediatamente con tapones de goma, se agitaron y se colocan en la incubadora a 39 ºC. El volumen de gas producido se registró en tiempos de 2, 4, 6, 8, 10, 12, 14, 24, 30, 48, 54 y 72 h de incubación, utilizando la técnica de lectura de la presión (instrumentos de Extech, Waltham, EE.UU.) de Theodorou et al. (1994). Al final de la incubación (es decir, 72 h), las botellas fueron destapadas, el pH se midió utilizando un medidor de pH (Conductronic pH15, Puebla, México) y el contenido de cada botella se filtró para obtener el residuo no fermentado para la determinación de la degradabilidad del sustrato. Después de registrar el

volumen final de gas (es decir, 72 h), se añadieron 2 ml de NaOH (10 M) a cada una de las botellas y la presión del gas se determinó de inmediato. La mezcla del contenido con NaOH permitió la absorción de CO2, con el volumen de gas que quedó en el espacio de superior de las botellas considerándolo como CH_4 (Demeyer et al., 1988).

Degradabilidad y análisis de la muestra

Al final de la incubación (es decir, 72 h), el contenido de cada botella de suero se filtró al vacío a través de crisoles de vidrio con un filtro sinterizado (porosidad gruesa no. 1, tamaño de poro 100 a 160 micras, Pyrex, Stone, UK). Los residuos de fermentación se secaron a 105ºC durante la noche para estimar la desaparición de la MS con la pérdida de peso después del secado siendo la medida de MS no degradable. La fibra detergente neutro (FDN) y la fibra detergente ácido (FDA) se determinaron en los residuos después de obtener la degradabilidad de la MS (DMS), para determinar la degradabilidad de la FDN y la FDA. La FDN se analizó sin el uso de alfa amilasa pero con sulfito de sodio. Tanto FDN y FDA se expresan sin ceniza residual. Las determinaciones de FDN y FDA también se hicieron en los residuos después de incubaciones para la degradabilidad de FDN y de FDA. Se analizaron muestras de los alimentos para MS (# 934.01), cenizas (# 942.05), N (# 954.01) y EE (# 920.39), según la AOAC (1997). La fibra detergente neutro (FDN, Van Soest et al., 1991), la fibra detergente ácido (FDA) y la lignina (AOAC, 1997; # 973.18) para los análisis se utilizó el equipo ANKOM200 Fibre Analyzer United (ANKOM Technology Corp., Macedonia, Nueva York, EE.UU.).

Cálculos y análisis estadísticos

Todos los cálculos se mencionaron y describieron antes en Salem (2012 de la siguiente manera:

Los parámetros cinéticos de la producción de gas (GP) se estimaron (ml/g MS) con datos ajustados en la opción NLIN de SAS (2002) de acuerdo con France et al. (2000) como sigue:

$A = b \times (1 - e^{-c\ (t-L)})$

Donde: A es el volumen de PG en el tiempo t; *b* es la asintótica de PG (ml/g MS); *c* es la velocidad de PG (/h), y *L* (h) es el tiempo de retraso antes de la producción de gas.

La energía metabolizable (ME, MJ/kg MS) y la digestibilidad *in vitro* de la materia orgánica (DMO, g/kg MO) se estimaron según Menke et al. (1979) como sigue:

EM = 2.20 + 0.136 PG (ml/0.5 g MS) + 0.057 PC (g/kg MS)

DMO = 148.8 + 8.89 PG + 4.5 PC (g/kg MS) + 0.651 cenizas (g/kg MS)

dónde: PG es la PG neta en ml de 200 mg de muestra seca después de 24 h de incubación.

El factor de reparto a las 24 h de incubación (PF_{24}, una medida de la eficiencia de la fermentación) se calculó como la relación de la degradabilidad de MS *in vitro* (DMS, mg) y el volumen (ml) de PG a las 72 h (es decir, DMS / total de gas producción (PG_{96})) de acuerdo con Blummel et al. (1997). El rendimiento de gas (GY_{24}) se calculó como el volumen de gas (ml gas/g MS) producido después de 24 h de incubación dividido por la cantidad de DMS (g) como sigue:

Rendimiento de gas (GY_{24}) = ml gas/g MS/g DMS

La concentración de ácidos grasos de cadena corta (AGCC) se calcularon de acuerdo con Getachew et al. (2002) como sigue:

AGCC (mmol/200 mg DM) = 0.0222 PG – 0.00425

Donde: PG es la producción neta de gas a las 24 h (ml/200 mg MS).

El diseño experimental para el análisis de la PG, la degradabilidad y los parámetros de fermentación ruminal *in vitro* fue un diseño completamente al azar teniendo en cuenta, como factores fijos, el tipo de forraje (S) y el nivel de *S. cerevisiae* (C) en el modelo lineal (Steel et al., 1997) dentro de cada método (M) de aplicación (directo o pre-incubación). Los datos de cada una de las tres corridas dentro de la misma muestra se promediaron antes del análisis estadístico. Los valores medios de cada muestra individual dentro de cada especie (tres muestras de cada uno) fueron utilizados como la unidad experimental. El modelo estadístico fue:

$Y_{ijklm} = \mu + Sj + C_k + M_l + (S \times C)_{jk} + (S \times M)_{jl} + (M \times C)_{lk} + (S \times M \times C)_{jkl} + E_{ijklm}$

dónde: Y_{ijk} = es cada observación de i-ésima especie fibrosa, (Si) cuando se incubaron en la j-ésima levadura (C_j; *S. cerevisiae*); μ es la media general; S_i (i = 1-4) es el efecto del alimento; C_j es el efecto de la dosis de levadura (j = 1-4); M_{jis} el método de aplicación (j = 1-2), $(S * C)_{ij}$ es la interacción entre el alimento y la dosis de levadura; $(S * M)_{jl}$ es la interacción entre los alimentos y los métodos de aplicación; $(S * M * C)_{jkl}$ es la interacción entre las tres variables de estudio (alimentos, la levadura y el método de aplicación) y E_{ijklm} es error experimental. Contrastes polinomiales lineares y cuadráticos se utilizaron para examinar las respuestas de los alimentos al incrementar los niveles de adición de *S. cerevisiae.*

ARTICULO 3.

Titulo:

In vitro gas and methane production of two mixed rations influenced by three different products of *Saccharomyces cerevisiae*

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Se utilizaron como sustrato dos raciones mixtas con dos niveles diferentes de PC al 13% (BPC) y al16% (APC) en base seca (Tabla 1) y se incubaron con tres dosis de diferentes productos de *Sc*.

Tres tipos de cultivos de *Sc* fueron probados a tres dosis (mg/g de MS de sustrato): 0 (sin; SC0), 2 (SC2) y 4 (SC4). Antes de iniciar los tratamientos se preparó una cantidad suficiente de solución de cada levadura en agua destilada a fin de obtener las dosis adecuadas en 1 ml de la solución.

Se utilizaron tres productos de *Sc* (Lesaffre Feed Additives, Toluca, México): (1) Biocell® contiene un mínimo garantizado de 2.0×10^{10} UFC /g de *Saccharomyces cerevisiae*. (2) Procreatin 7® contiene un mínimo garantizado de 1.5×10^{10} UFC / g de *Saccharomyces cerevisiae*. (3) Biosaf SC47® contiene un recuento mínimo de células de levaduras vivas de 1.0×10^{10} UFC / g de *Saccharomyces cerevisiae*.

Incubaciones *in vitro*

Como fue descrito antes en Elghandour et al. (2014), tres ovejas (35 a 45 kg de peso corporal) con cánula ruminal permanente fueron utilizados como donantes de inóculo ruminal y se alimentaron con una ración integral de concentrado comercial y ensilado de maíz a proporción 1:1 MS formulado para cubrir sus necesidades de nutrientes (NRC 1985). Las ovejas tenían libre acceso a agua fresca durante todo el tiempo de la fase de obtención del inóculo ruminal.

Antes de la alimentación de la mañana, se obtuvo contenido ruminal de cada oveja y se gaseó con CO_2 para mantenerlo en condiciones anaerobias, a continuación se mezcló y filtró a través de cuatro capas de gasa en un matraz con espacio superior libre de O_2. Las muestras de los alimentos (0,5 g) se pesaron en frascos de suero de 120 ml con la adición apropiada de la dosis de levadura /g MS. Se añadieron diez ml de fluido ruminal libre de partículas a cada botella seguido de 40 ml de la solución tampón de acuerdo con Goering y Van Soest (1970), sin agregar tripticasa, en una proporción 1: 4 (vol / vol).

Una vez que todas las botellas estaban llenas, fueron cerradas inmediatamente con tapones de goma, se agitaron y se colocaron en la incubadora a 39 ºC. El volumen de gas producido y la producción de CH4 se registraron a tiempos de 2, 4, 6, 8, 10, 12, 14, 24, y 48 h de incubación. La producción de gas se registró usando la técnica del lector de presión (Extech instruments, Waltham, EE.UU.) de Theodorou et al. (1994), mientras que la emisión de metano fue registrada usando el Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK).

Después de 48 h de incubación, las botellas fueron destapadas, el pH se midió utilizando un medidor de pH y el contenido de cada botella se filtró para obtener el residuo no fermentado para la determinación del sustrato degradado.

Degradabilidad y análisis de la muestra

La degradabilidad y los análisis se determinaron como se describió en Elghandour et al. (2014). Brevemente, después de 48 h de incubación, se detuvo el proceso de

fermentación y el contenido de cada botella se filtró al vacío a través de crisoles de vidrio con un filtro sinterizado. Los residuos de fermentación se secaron a 105 ° C durante la noche para estimar la desaparición de MS. Tanto la FDN como la FDA se determinaron en los residuos después de calcular la degradabilidad de la MS (DMS) determinando también la degradabilidad de la FDN (DFDN) y FDA (DFDA). Se analizaron muestras de los alimentos para MS (# 934.01), cenizas (# 942.05), N (# 954.01) y EE (# 920.39), según la AOAC (1997). El contenido de FDN y FDA de ambos alimentos y sus residuos de fermentación se determinó a través del equipo ANKOM200 Fibre Analyzer Unit (ANKOM Technology Corp., Macedonia, Nueva York, EE.UU.) sin el uso de alfa amilasa pero con sulfito de sodio en la FDN (Van Soest et al. 1.991). Tanto FDN y FDA se expresan sin ceniza residual.

Cálculos y análisis estadísticos

Como fu descrito antes en Salem et al. (2014b), para calcular los parámetros cinéticos de PG, los resultados (ml / g MS) se ajustaron mediante la opción NLIN de SAS (2002) de acuerdo con el modelo de France et al. (2000) como sigue:

$A = b \times (1 - e^{-c\ (t-L)})$

Dónde: A es el volumen de PG en el tiempo t; *b* es la asíntota de PG (ml/g MS); *c* es la velocidad de PG (/ h), y *L* (h) es el lapso de tiempo antes de la PG.

La energía metabolizable (ME, MJ/kg MS) y la digestibilidad *in vitro* de la materia orgánica (DMO, g/kg MO) se estimaron según Menke et al. (1979) como sigue:

EM = 2.20 + 0.136 PG (ml/0.5 g MS) + 0.057 PC (g/kg MS)

DMO = 148.8 + 8.89 PG + 4.5 PC (g/kg MS) + 0.651 cenizas (g/kg MS)

Dónde: PG es la PG neta en ml de 200 mg de muestra seca después de 24 h de incubación.

El factor de reparto a las 24 h de incubación (PF_{24}, una medida de la eficiencia de la fermentación) se calculó como la relación de la degradabilidad de MS *in vitro* (DMS, mg) y el volumen (ml) de PG a las 24 h (es decir, DMS / total de gas producción (PG_{24})) de acuerdo con Blummel et al. (1997). El rendimiento de gas (GY_{24}) se calculó como el volumen de gas (ml gas/g MS) producido después de 24 h de incubación dividido por la cantidad de DMS (g) como sigue:

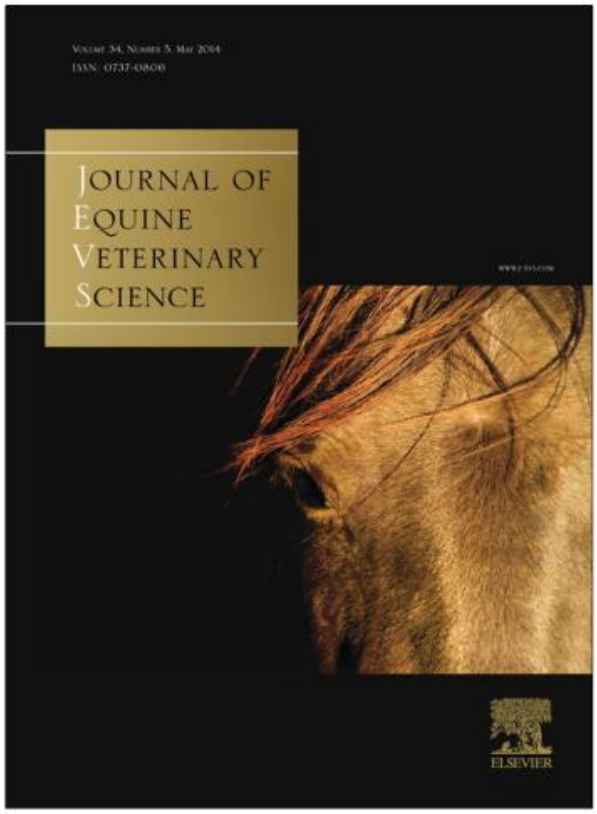
Rendimiento de gas (GY_{24}) = ml gas/g MS/g DMS

El diseño experimental para la producción de gas (PG), el análisis de las emisiones de metano, la degradabilidad y los parámetros de fermentación ruminal *in vitro* fue completamente al azar considerando como factores fijos, el tipo de ración (R) y las dosis de levadura (D) en el modelo lineal (Steel et al . 1.997) dentro de cada producto de levadura (P). Los datos de cada una de las tres corridas de la misma muestra se promediaron antes del análisis estadístico. Los valores de cada muestra individual dentro de cada especie (tres muestras de cada uno) fueron utilizados como la unidad experimental. El modelo estadístico fue:

$Y_{ijkl} = \mu + R_i + D_j + P_k + (R * D)_{ij} + (R * P)_{ik} + (P * D)_{jk} + (R * P * D)_{ijk} + E_{ijkl}$

dónde: Y_{ijkl} = es cada observación de la i-ésima ración (R_i) cuando se incubó al j-ésimo nivel D_j; μ es la media general; R_i (i = 1-2) es el efecto de la ración; D_j es el efecto dosis de levadura (j = 1-3); P_k es el tipo de levadura (j = 1-3), $(R * D)_{ij}$ es la interacción entre la ración y la dosis de levadura; $(R * P)_{jk}$ es la interacción entre la ración y la tipo de levadura; $(R * P * D)_{ijk}$ es la interacción entre la ración, el tipo de levadura y las dosis; y E_{ijkl} es error experimental. Contrastes polinomiales lineares y cuadrático se utilizaron para examinar las respuestas de las raciones a dosis crecientes de adición de los tipos de levadura..

VII.- RESULTADOS



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Original Research

In Vitro Fermentative Capacity of Equine Fecal Inocula of 9 fibrous Forages in the Presence of Different Doses of *Saccharomyces cerevisiae*

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ABSTRACT

This experiment was conducted to evaluate *in vitro* effects of equine fecal inocula fermentative capacity on 9 fibrous forages in the presence of *Saccharomyces cerevisiae*. The fibrous feeds were corn stover (*Zea mays*), oat straw (*Avena sativa*), sugarcane bagasse and leaves (*Saccharum officinarum*), llanero grass leaves (*Andropogon gayanus*), Taiwan grass leaves (*Pennisetum purpureum*), sorghum straw (*Sorghum vulgare*), and steria grass leaves (*Cynodon plectostachyus*). Fibrous feed samples were incubated with several doses of *S. cerevisiae*; 0 (control), 1.25 (low), 2.5 (medium) and 5 (high) mg/g dry matter (DM) of a commercial yeast product containing 1×10^{10}/g. Fecal inoculum was collected from 4 adult horses were fed on an amount of commercial concentrate and oat hay ad libitum. Gas production (GP) was recorded at 2, 4, 6, 8, 10, 12, 24, and 48 hours post inoculation. An interaction occurred between feeds and yeast dose for fecal pH ($P < .01$), asymptotic GP (*b*, ml/g DM); rate of GP (*c*, /hr); initial delay before GP began (*L*, hours), GP at 4 hours and 48 hours ($P < .01$), and GP at 8 hours ($P < .01$) and at 24 hours ($P < .01$). Differences in fecal fermentation capacity between the tropical and template grass ($P < .05$) occurred for fecal pH, *c*, and GP during first 12 hours, whereas differences occurred ($P < .05$) between the agriculture byproducts and the grasses for fecal pH, *b*, and GP from 8 to 48 hours. Fermentation capacity between straws versus not straws ($P < .05$) differed for fecal pH, *b*, and GP after 12 hours between straws *versus* not straws. Addition of *S. cerevisiae* to *Z. mays* stover reduced ($P < .01$) fecal pH and the *c* fraction with a higher ($P < .01$) *b* fraction *versus* the other feeds. From 4 to 24 hours, *S. officinarum* bagasse improved GP to the highest values *versus* *S. officinarum* leaves. After 24 hours, *Z. mays* stover had the highest GP, whereas *C. plectostachyus* leaves had the lowest. There were no differences among the yeast doses for all measured parameters with the exception of *L* values (linear effect; $P < .01$). The *Z. mays* stover had the highest nutritive compared to the other fibrous feeds. However, addition of *S. cerevisiae* at 2.5 to 5.0 g/kg DM improved fecal fermentation capacity of low-quality forages.

1. Introduction

The presence of microorganisms in the hindgut allows the horse to efficiently digest fiber and roughage and often

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leads to their selection as the main component of mature horse diets [1]. Although the horse is able to nutritionally use diets high in fibrous feeds, their digestive system is not as efficient as that of ruminants [1].

There is increasing interest in feeding fiber-based feeds as an alternative to high-starch cereal grains to horses as a means of meeting the energy demands and reduce various pathologies, such as gastric ulceration, hind-gut acidosis, laminitis, and colic [2,3], associated with feeding high levels of cereal grains. Depending on the forage type and time of harvest, forages of moderate to high nutritive value may meet the energy and crude protein (CP) requirements of horses [4]. Horses naturally use forage as a primary component of their diet, and forage is a basic necessity for normal function of the equine digestive system. High forage rations are desirable because they contain low levels of starch and sugar. Feeding a minimum 1% of body weight as fiber is very important to minimize the incidence of hindgut acidosis [5], colic [6], gastric ulcers [7], and stereotypical behaviors [8]. In tropical areas such as Mexico, forages used as feeds are generally low in digestibility and low in true protein [9]. Therefore, there is a need to develop feeding strategies which meet the requirements of performance horses while maintaining gut health and integrity.

Yeast cultures can increase the number of lactate-using bacteria and result in increased cecal pH [10]. In some studies, supplementation of equine diets with a yeast culture has improved digestion of low-quality forages, which would be advantageous to the horse's health [11]. Yeast cultures can provide enhanced microbial environmental conditions and/or increase the total number of hindgut microorganisms, resulting in improved digestibility of forages in the hindgut [1].

However, previous research results with *Saccharomyces cerevisiae* supplementation of horse diets has been variable and inconsistent. Addition of *S. cerevisiae* to equine diets may stimulate hindgut digestion by altering microbial population, and some research has indicated that adding *S. cerevisiae* to the diet of equines can improve nutrient digestibility, [12] increase microbial populations [13,14], and maintain cecal pH [13,15]. However, other studies have reported no improvement in nutrient digestibility when *S. cerevisiae* supplements were given to horses *in vivo* [16,17] and *in vitro* [14].

The purpose of this experiment was to evaluate the fermentative capacity of 9 fibrous forages in the presence of various doses of *S. cerevisiae*, using the *in vitro* gas production (GP) technique of Theodorou et al [18] as an indicator of hindgut activity using equine fecal inocula.

2. Materials and Methods

2.1. Fibrous Feed Species and Yeast Culture Levels

Three individual samples of each of the fibrous feeds of corn stover and cobs (*Zea mays*), oat straw (*Avena sativa*), sugarcane bagasse and leaves (*Saccharum officinarum*), llanero grass leaves (*Andropogon gayanus*), Taiwan grass leaves (*Pennisetum purpureum*), sorghum straw (*Sorghum vulgare*), and steria grass leaves (*Cynodon plectostachyus*) were randomly and manually harvested in triplicate from different sites in the state of Mexico in Mexico. Samples of stover, straws, and leaves were collected at the last stages of maturity and dried at 60°C for 48 hours in a forced air oven to constant weight, ground in a Wiley mill to pass through a 1-mm sieve, and stored in plastic bags for subsequent determination of chemical components and *in vitro* GP. Four levels of Saccharomyces *cerevisiae* commercially available as a feed additive (Procreatin 7; Safmex/Fermex S.A. de C.V., Toluca, Mexico) in powdered form containing 1×10^{10} colony-forming units (CFU)/g of yeast product were used at levels (mg/g dry matter [DM] of substrate) of control (0 mg), low (1.25 mg), medium (2.50 mg), and high (5.00 mg). Feed samples were incubated with yeast doses which were added to the bottles immediately before incubation. A stock solution of yeast culture doses was prepared before treatments in distilled water in order to obtain suitable doses in each 1 ml of stock solution.

2.2. In Vitro Incubation

Fecal inoculum was collected from 4 adult horses ranging from 5 to 8 years of age and weighing 480 ± 20.1 kg. Horses were fed daily on an amount of commercial concentrate (Purina, Toluca, Mexico) and oat hay as ad libitum. Fresh water was available to the horses at all times.

The methods used for the GP technique were as described by Theodorou et al [18]. Fecal contents were collected directly from the rectum of each horse and immediately transferred to the laboratory for *in vitro* incubation. Fecal contents were combined with culture medium in a ratio of 1:4 and kept dispensed under CO_2 immediately after extraction and during the incubation process. Fecal inoculum mixed with culture medium was used to inoculate 3 identical series (runs) of bottles containing 1 g of DM of each 1 of the fibrous feed species as substrates. For each inoculum, 3 substrate-negative controls (blank) were also included. This resulted in a total of 324 bottles for GP (9 fibrous feeds × 3 individual samples × 3 runs × 4 yeast doses). Once all bottles were filled, they were immediately closed with rubber stoppers, shaken, and placed in the incubator at 39°C. Gas production readings were made at 2, 4, 6, 8, 10, 12, 24, and 48 hours post-inoculation, using the pressure reading technique (Extech instruments; Waltham, CT, USA). At the end of incubation (ie, 72 hours), bottles were uncapped, and pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico).

2.3. Calculations and Statistical Analyses

To estimate kinetic parameters of GP, results (ml/g DM) were fitted using the NLIN option of SAS [19] according to France et al [20] as:

$$A = b \times (1\, e^{-c(t-L)})$$

where: A is the volume of GP at time t; b is the asymptotic GP (ml/g DM); c is the rate of GP (/hr), and L (hours) is the discrete lag time prior to GP.

The experimental design was a completely randomized design considering, as fixed factors, feed species (S) and

Table 1
Sample types and chemical compositions of the 9 fibrous feeds (g/kg DM)

Species	Sample	Organic Matter	Crude Protein	Acid Detergent Fiber	Neutral Detergent Fiber
Zea mays	Straw-tropical	959.7	62.9	274.4	476.7
Avena sativa	Straw- tropical	923.6	37.2	380.0	537.8
Zea mays	Cobs leaves- tropical	976.1	21.4	428.9	698.9
Saccharum officinarum	Bagasse- tropical	982.0	25.7	324.4	458.9
Saccharum officinarum	Leaves- tropical	929.7	42.9	385.6	614.4
Andropogon gayanus	Leaves- temperate grass	948.5	22.9	485.2	697.8
Pennisetum purpureum	Leaves- temperate grass	948.5	22.9	482.2	697.8
Sorghum vulgare	Straw- tropical	944.3	40.0	377.8	556.7
Cynodon plectostachyus	Leaves- temperate grass	912.2	42.0	362.2	584.4

yeast culture doses (D) in the linear model [21]. Data from each of the 3 runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample within each species (ie, 3 samples of each) were used as the experimental unit. The statistical model was:

$$Y_{ijk} = \mu + S_j + D_k + (S \times D)_{jk} + E_{ijk}$$

where: Y_{ijk} = is every observation of the ith fibrous specie (S_j) when incubated in the jth yeast (D_k; yeast culture doses); μ is the general mean; S_j (j = 1 to 9) is the feed effect; D is the yeast doses effect (k = 1 to 4); $(S \times D)_{jk}$ is the interaction between feed and yeast dose; and E_{ijk} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses of feeds to increasing addition levels of the yeast culture.

3. Results

3.1. Chemical Composition

Chemical analysis showed that the organic matter (OM) did not differ and ranged between 912 and 982 g/kg DM for *C. plectostachyus* leaves and *S. officinarum* bagasse, respectively. Corn stover (*Z. mays*) had the highest ($P < .05$) CP and the lowest ($P < .05$) acid detergent fiber (ADF) and neutral detergent fiber (NDF). However, both the leaves of *Z. mays* and *P. purpureum* had the highest ($P < .05$) ADF and NDF and the lowest ($P < .05$) CP (Table 1).

3.2. In Vitro Gas Production

Both the fibrous species and yeast dose effects had an interaction for fecal pH ($P < .01$), asymptotic GP (b, ml/g DM); rate of GP (c, /hr); initial delay before GP began (L, hours) and GP at 4 and 48 hours ($P < .01$). Interactions occurred also for GP at 8 ($P < .01$), and 24 hours ($P < .01$).

Contrasting effects of fecal fermentation capacity occurred between tropical and temperate grasses for fecal pH ($P < .00$), c ($P < .05$), GP during the first 12 hours ($P < .05$) and also occurred for fecal pH ($P < .01$), b ($P < .01$), and GP from 8 to 48 hours ($P < .05$) between agriculture byproducts and grasses. Straw and no-straw fibrous feeds had differences in their fermentation for fecal pH ($P < .01$), b ($P < .01$), and GP after 12 hours ($P < .05$).

Fermentation of *Z. mays* stover with *S. cerevisiae* reduced ($P < .01$) fecal pH and the rate of GP (/hr), but asymptotic GP (b, ml/g DM) was higher ($P < .01$) than in the other fibrous species. In contrast, *C. plectostachyus* leaves had the lowest ($P < .01$) asymptotic GP with the highest ($P < .01$) rate of GP (c, /hr). The *S. officinarum* bagasse reduced ($P < .01$) the fermentation lag to its lowest level compared to *P. purpureum* leaves, which had ($P < .01$) the highest values. During the period from 4 to 24 hours, *S. officinarum* bagasse improved *in vitro* GP to maximum recorded values compared to *S. officinarum* leaves, which had the lowest values. After 24 hours (ie, GP between 24 and 48 hours), *Z. mays* stover had the highest, whereas *C. plectostachyus* leaves had the lowest *in vitro* GP. The other species varied in terms of *in vitro* GP, with intermediate values at different times.

No effects were observed among yeast doses for fecal pH, the asymptotic GP (b, ml/g DM), the rate of GP (c, /hr), and *in vitro* GP at any measured time. Addition of *S. cerevisiae* caused varied responses for the initial delay before GP began between fibrous species. However, addition of *S. cerevisiae* linearly reduced ($P < .01$) the initial delay before GP began for *Z. mays* stover (linear effect, $P < .01$; quadratic effect, $P = .07$), *S. officinarum* bagasse (linear effect, $P < .01$), and leaves (linear effect, $P < .05$), *P. purpureum* leaves (linear effect, $P < .01$; quadratic effect, $P < .05$), and *C. plectostachyus* leaves (linear effect, $P < .01$; quadratic effect, $P < .05$) compared to the other fibrous species, which increased with a dose of 1.25 mg/g DM for *A. sativa* straw (linear effect, $P < .05$; quadratic effect, $P < .01$) and *A. gayanus* (quadratic effect, $P < .01$) leaves (Table 2). Effect of different *S. cerevisiae* doses with different fibrous species was negligible for *in vitro* GP (mL/g DM) (Table 2).

4. Discussion

Based on chemical composition and *in vitro* fermentation kinetics of our fibrous feeds, the tropical species had higher nutritive value than the temperate grasses. The same occurred where agriculture byproducts had a higher nutritive value than grasses. Moreover, straw had higher nutritive value than the not straws. However, many studies have shown that temperate grasses have advantages over subtropical grasses, including a higher nutritive value. Temperate grasses generally have a higher DM digestibility, due primarily to lower lignin content [22]. Compared with temperate forages, tropical forages typically have increased annual DM yield, although this increased yield is usually associated with decreased forage quality [23]. These differences may be based on geographic regions or unknown factors.

Although the technique of Theodorou et al [18] for studying *in vitro* fermentation initially relied upon rumen

Table 2
In vitro fecal gas kinetics of nine low quality roughages as affected by different levels of *Saccharomyces cerevisiae* (mg/g DM)

Species	Fibrous Species	Fecal pH	Gas Production Parameters[a]			*In Vitro* Gas Production (ml/g DM)					
			b	*c*	*L*	Gas4	Gas8	Gas10	Gas12	Gas24	Gas48
Zea mays (stover)	0	6.5	202.4	0.024	2.57	26.0	48.3	70.2	83.7	129.4	171.0
	1.25	6.5	182.3	0.030	1.77	30.4	55.7	79.9	94.4	139.8	172.4
	2.5	6.6	191.4	0.025	1.64	26.6	49.5	72.2	86.2	133.6	173.9
	5.0	6.6	190.2	0.025	1.78	26.4	49.1	71.6	85.4	132.3	172.3
	P value										
	Linear	0.002	0.161	0.769	0.001	0.895	0.870	0.841	0.824	0.755	0.745
	Quadratic	0.4723	0.045	0.153	0.070	0.286	0.306	0.335	0.353	0.478	0.993
Avena sativa (straw)	0	6.8	115.0	0.051	2.19	30.2	52.4	71.1	80.9	104.8	114.1
	1.25	6.8	115.2	0.051	2.73	30.3	52.6	71.3	81.1	105.1	114.3
	2.5	6.8	115.3	0.050	1.57	29.8	51.9	70.6	80.4	104.7	114.3
	5.0	6.8	113.5	0.048	1.68	28.4	49.7	67.7	77.4	101.8	112.2
	P value										
	Linear	.860	.948	.821	.015	.857	.883	.899	.908	.979	.963
	Quadratic	.760	.987	.965	.001	.904	.887	.895	.894	.922	.978
Zea mays (cobs leaves)	0	6.8	94.50	0.053	1.32	24.1	41.6	56.3	64.0	83.4	92.6
	1.25	6.8	139.2	0.025	1.43	19.5	36.3	53.0	63.2	97.7	126.8
	2.5	6.8	89.49	0.048	1.83	22.0	38.5	52.7	60.3	79.8	88.4
	5.0	6.8	154.2	0.030	1.45	25.1	45.4	64.4	75.5	110.7	139.0
	P value										
	Linear	.291	.686	.656	.079	.701	.730	.752	.763	.780	.727
	Quadratic	.052	.002	.028	.538	.468	.631	.876	.922	.174	.008
Saccharum officinarum (bagasse)	0	6.7	119.6	0.090	2.50	50.0	79.1	98.0	105.8	118.0	119.6
	1.25	6.7	116.6	0.131	1.81	57.3	88.3	104.2	109.5	116.0	116.6
	2.5	6.7	143.5	0.087	1.40	57.2	91.6	114.7	124.5	140.9	143.5
	5.0	6.8	115.7	0.075	0.72	40.3	67.6	87.3	96.1	112.5	115.6
	P value										
	Linear	.003	.048	.861	.008	.044	.023	.016	.021	.041	.047
	Quadratic	.044	.129	.019	.628	.193	.023	.658	.344	.139	.129
Saccharum officinarum (leaves)	0	6.9	90.47	0.039	2.50	18.6	33.1	46.3	53.6	74.7	87.3
	1.25	7.0	103.0	0.030	1.63	16.2	29.7	42.7	50.4	75.4	94.8
	2.5	7.0	104.7	0.029	1.50	15.9	29.3	42.2	49.9	74.9	94.9
	5.0	7.0	87.37	0.047	1.58	21.1	37.1	50.9	58.3	77.5	86.2
	P value										
	Linear	.291	.164	.204	.035	.302	.334	.371	.422	.963	.110
	Quadratic	.587	.520	.535	.313	.625	.652	.690	.737	.809	.346
Andropogon gayanus (leaves)	0	6.9	127.3	0.030	1.90	20.7	38.1	54.8	64.7	96.3	119.6
	1.25	6.8	131.6	0.027	2.83	19.6	36.1	52.3	62.1	94.4	120.6
	2.5	6.8	116.3	0.04	1.70	23.7	42.3	58.9	68.3	95.0	111.5
	5.0	6.8	118.3	0.033	1.49	21.5	39.1	55.6	65.3	94.5	113.5
	P value										
	Linear	.135	.240	.196	.550	.241	.272	.322	.380	.652	.200
	Quadratic	.236	.225	.241	.006	.230	.218	.218	.221	.608	.341
Pennisetum purpureum (leaves)	0	6.9	88.97	0.052	2.73	22.1	38.2	51.7	58.8	77.1	86.6
	1.25	7.0	104.7	0.045	2.42	24.3	42.7	58.7	67.4	90.6	102.3
	2.5	6.9	95.60	0.051	1.43	22.3	38.6	52.4	59.8	79.6	91.4
	5.0	7.0	120.7	0.034	1.04	19.2	34.6	49.1	57.6	84.6	106.9
	P value	.691	.803	.918	<.0001	.960	.922	.891	.863	.821	.811
	Linear	.191	.592	.662	.031	.461	.314	.194	.1560	.230	.452
	Quadratic										
Sorghum vulgare (straw)	0	6.8	124.2	0.038	2.50	24.2	43.4	61.0	71.1	100.5	119.0
	1.25	6.7	120.9	0.032	1.92	20.3	37.0	52.7	62.0	91.0	112.4
	2.5	6.7	100.4	0.044	1.63	21.5	37.9	52.2	60.2	82.8	96.6
	5.0	6.7	53.73	0.377	0.80	45.4	52.1	53.4	53.6	53.7	53.7
	P value										
	Linear	.361	.234	.921	.001	.453	.283	.201	.165	.143	.202
	Quadratic	.641	.607	.869	.381	.418	.405	.493	.568	.946	.747
Cynodon plectostachyus (leaves)	0	6.8	51.90	0.202	2.58	35.6	46.4	50.2	51.1	51.9	51.9
	1.25	6.8	61.93	0.117	1.86	29.0	43.2	51.6	54.8	60.5	61.9
	2.5	6.8	60.27	0.167	1.52	38.2	52.1	57.7	59.2	60.3	60.3
	5.0	6.8	59.23	0.121	1.08	30.1	44.7	52.7	55.4	58.9	59.2
	P value										
	Linear	.863	.153	.329	<.0001	.489	.089	.014	.016	.099	.150
	Quadratic	.335	.238	.048	.016	.031	.046	.282	.893	.285	.239
LSD pooled		0.052	8.654	0.0404	0.397	5.45	8.22	10.23	11.24	12.06	10.24
P value:											
Fibrous species		<.0001	<.0001	<.0001	.0004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

(*continued on next page*)

Table 2 (*continued*)

Species	Fibrous Species	Fecal pH	Gas Production Parameters[a]			*In Vitro* Gas Production (ml/g DM)					
			b	*c*	*L*	Gas4	Gas8	Gas10	Gas12	Gas24	Gas48
Yeast doses:											
Linear		.865	.946	.612	<.0001	.585	.470	.460	.474	.561	.688
Quadratic		.595	.079	.281	.168	.399	.735	.987	.840	.247	.059
Fibrous species × yeast doses		.001	<.0001	<.0001	<.0001	<.0001	.005	.103	.090	.001	<.0001
Contrasts effects:											
Tropical vs. temperate grasses		<.0001	.831	.032	.201	.001	.002	.009	.022	.274	.710
Agriculture byproducts vs. grasses		<.0001	.0001	.307	.360	.079	.006	.002	<.0001	<.0001	<.0001
Straws vs. no straws		<.0001	<.0001	.968	.259	.920	.547	.138	.036	.002	<.0001

DM, dry matter; LSD, least significant difference.
[a] *b* is the asymptotic gas production (ml/g DM); *c* is the rate of gas production (/hr); *L* is the initial delay before gas production begins (hours).

fluid as the source of microbial inoculum, use of feces as the source of microbial inoculum has proved to be a successful alternative source of microbial inoculum in ruminants [14,24-26] and equine [27-30] studies. Use of inocula for *in vitro* GP from either rumen fluid or feces resulted in few differences in accumulation of gas, although the lag phase appeared to be higher when feces were used. This may be due to the different number of microorganisms per gram of rumen digesta or feces. A similar situation is evident in equines, wherein studies have shown a similar pattern of GP using feces fluid or equine feces as the inoculum but with a lower difference in the lag phase noted between cecal fluid and feces compared than that seen with rumen fluid and feces [30,31]. Microorganisms such as bacteria, protozoa, and fungi are found in the hindgut [32] and are similar to the microbes in the rumen [33]. However, bacteria and fungi seem to play a much bigger role in fiber digestion than protozoa do [32].

Use of *in vitro* fermentation procedures to study diet digestion and fermentative end products has become increasingly popular in equine nutrition, based on validation of the use of equine feces as the source of inoculum. Macheboeuf and Jestin [34] and Lowman et al [35] have shown that grains and forages incubated with equine feces produced GP profiles similar to known gas concentrations. Furthermore, Ringler et al [36,37] found that combined use of equine fecal inoculum yielded valid *in vitro* estimates of DM, NDF, and ADF digestibility.

Fibrous feeds can be the main sources of nutrients for equines for long periods of time, especially during high-latitude winters and in the dry season when resources are in short supply [38]. The chemical composition of different fibrous species and varieties varies widely, but there are many factors which cause this variation. The most important factor is the growing conditions, such as the genotype of the crops, differences among production environments, and the interaction between environment and genotypes [35]. Environmental differences include variations in climate, soil, and agronomic practices. In addition, variations arise from differences in harvesting conditions and postharvesting treatments [39,40]. In contrast, pasture management techniques cannot be ignored [41], as there is usually an inverse relationship between the CP and fiber content in a forage species [42], and this occurred in our study where the CP content was highest with *Z. mays* stover, *S. officinarum* leaves, and *A. gayanus* leaves where low contents of both NDF and ADF occurred, but vice versa, in the case of *Z. mays* cobs leaves and Taiwan grass. This phenomenon will affect the asymptotic GP and *in vitro* GP with advancing times of incubation.

Responses to the addition of dietary *S. cerevisiae* are dependent upon yeast source, fibrous species type, forage composition, application method, and dose-dependent interactions between yeast and diet [43,44]. In our study, the same yeast with the same doses and application methods resulted in different responses to the different fibrous feeds. Production of gases from the tested roughages depends on the CP and fiber contents of the feeds [45]. The different substrates used in our study caused different individual fermentation characteristics as to process dynamics and products while incubated with equine feces. As the volume of GP reflects the fermentation potential of the fiber fraction [26], the linearly higher GP during the first period of fermentation (ie, during the first 12 hours) of both *S. officinarum* bagasse and leaves refers to its high content of highly fermentable constituents versus the other fibrous feeds. In contrast, the fermentation process of *Z. mays* stover is dependent upon their content of slowly fermented constituents; GP depends on nutrient availability for inocula microorganisms [46].

Addition of yeast culture had a positive overall effect on GP from most of the substrates. Yeast supplementation is likely to stimulate the microbial cellulolytic activity in the hindgut causing an improved fiber digestion [47]. Previous studies indicated that live yeasts can improve the microbial balance in the hindgut of horses, stimulating the population of cellulolytic bacteria and their activity [13], thereby increasing the digestibility of dietary nutrients [48,49]. Lattimer et al [14] suggested that yeast culture supplementation resulted in improved energetics of the microflora and, as a result of the improved the microbial balance in the hindgut with stimulated cellulolytic bacteria activity and increased digestibility, the amount of gas produced increased.

However, diet composition-related ability of live yeasts to modify microbial digestion and fiber degradation in horses has not been extensively studied [47]. The DM, NDF, and ADF digestibilities were enhanced in mature horses fed

a forage diet supplemented with yeast culture [48], and McDaniel et al [50], using cecal fluid from mature horses consuming a high-fiber diet, reported an increase in the acetate-to-propionate ratio as well as in the total volatile fatty acids (VFA) concentrations *in vitro* [10].

A shorted fermentation lag time with yeast addition is due to 2 basic mechanisms. The first was reported by Newbold et al [51] as the respiratory activity which scavenges O_2, which is toxic to anaerobic bacteria and causes inhibition of adhesion of cellulolytic bacteria to cellulose, and this peak in O_2 concentration occurs at approximately the time of feeding. The second mode of action is based on the fact that yeast contain small peptides and other nutrients which are required to stimulate ruminant cellulolytic bacteria to initiate growth [52,53]. In our study, addition of *S. cerevisiae* shortened the lag time to first GP compared to control (ie, 0 mg of *S. cerevisiae*/g DM). However, increasing doses of *S. cerevisiae* in most fibrous feeds decreased the lag time.

Effects of feeding yeast culture on fecal pH depend upon the fermented substrate. Because increased lactate concentrations are known to lower the pH and maintain a more desirable pH in the cecum [15] and thus increase fiber digestion, removal of excess lactate is beneficial. Jouany et al [47] showed an increase in lactate utilizing bacteria in the caecum of animals [54]. In some studies, there was a trend toward a higher cecal pH in horses fed the yeast culture, and *S. cerevisiae* supplementation appeared to minimize the level of adverse changes to pH in the cecum of the horse [15]. The response to increasing *S. cerevisiae* doses varied among our fibrous feeds, but, in general, desirable effects occurred with doses of 2.5 to 5.0 g yeast/kg DM in most of the fibrous feeds.

5. Conclusions

Addition of live yeast (*S. cerevisiae*) to 9 different fibrous feeds resulted in different *in vitro* GP from these substrates, which, if a similar scenario exists *in vivo*, has important implications for the overall energy balance of the equines. Based on the highest asymptotic GP and CP content occurring with the lowest contents of both NDF and ADF, *Z. mays* stover had the highest nutritive value compared to the other fibrous feeds. Low or nonexistent effects of *S. cerevisiae* addition on *in vitro* fecal gas kinetics of some feeds improved fecal fermentation kinetics with the other forages at 2.5 to 5.0 g/kg DM. Based on chemical composition and *in vitro* fermentation kinetics, higher nutritive values occurred with tropical species than with temperate grasses, with agricultural byproducts than grasses, and with straws than not straws.

Nevertheless, further research is required to elucidate the mode of action of *S. cerevisiae* and to investigate factors such as yeast supplementation level, substrate, application methods used, and yeast/substrate interactions on cecal fermentation and *in vivo* nutrient digestibility.

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PAPER

Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on *in vitro* gas production kinetics and degradability of four fibrous feeds

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Abstract

The objective of this study was to evaluate the effects of *Saccharomyces cerevisiae* on *in vitro* gas production (GP) kinetics and degradability of corn stover, oat straw, sugarcane bagasse and sorghum straw. Feedstuffs were incubated with different doses of yeast [0, 4, 8 and 12 mg/g dry matter (DM)] at direct addition or 72 h pre-incubation. Rumen GP was recorded at 2, 4, 6, 8, 10, 12, 14, 24, 30, 48, 54 and 72 h of incubation. After 72 h, rumen pH and methane were determined and contents were filtrated for DM, neutral (NDF) and acid detergent fibre (ADF) degradability. Fibrous species×method of application×yeast interactions occurred ($P<0.001$) for all measured ruminal GP parameters and degradability. The direct addition or 72 h pre-incubation of *S. cerevisiae* with corn stover improved ($P<0.05$) GP and methane and decreased ($P<0.05$) the lag time (*L*) and NDF degradability (NDFD). The direct addition of *S. cerevisiae* to oat straw increased ($P<0.05$) rate of GP (*c*) and decreased ($P<0.05$) asymptotic GP (*b*). However, 72 h pre-incubation increased ($P<0.05$) *c* with linearly decreased *b*, DM degradability (DMD) and NDFD. Applying *S. cerevisiae* for 72 h pre-incubation decreased ($P<0.001$) methane emission. The direct addition or 72 h pre-incubation of *S. cerevisiae* to sorghum straw increased ($P<0.05$) *b*, *c*, *L*, DMD and NDFD. Overall, the effect of dose varied among different feedstuffs and different application methods. Results suggested that the direct addition of *S. cerevisiae* could support and improve ruminal fermentation of low-quality forages at 4 to 12 g/kg DM.

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Introduction

Direct-fed microbial offer a great potential for manipulation of ruminal fermentation and *Saccharomyces cerevisiae* is an especially attractive organism. *S. cerevisiae* addition was reported to increase nutritional value of poor quality forages. *S. cerevisiae* have the ability to increase dry matter (DM) and neutral detergent fibre (NDF) digestion (Carro *et al*., 1992), increase initial rates of fibre digestion (Williams *et al*., 1991). Numerous studies (Kumar *et al*., 2013; Pinloche *et al*., 2013) documented positive effects of yeast, not only on the rumen environment, but also on the improvement of microbial activities. *S. cerevisiae* supplementation leads to increase in the number of total anaerobic and cellulolytic bacteria (Newbold *et al*., 1996; Jouany, 2001). *Saccharomyces cerevisiae* can provide the rumen with important nutrients and nutritional cofactors in addition to vitamins such as biotin and thiamine, which reported to be required for microbial growth and activity (Callaway and Martin, 1997; Mao *et al*., 2013).

Many reports illustrated that administration of yeast is paralleled with increased gas production (GP). Many studies suggested that *S. cerevisiae* might stimulate the acetogens to compete or co-metabolise hydrogen with methanogens, thereby reducing CH_4 emissions (Hristov *et al*., 2013). However, others reported increased CH_4 production (Martin *et al*., 1989; Martin and Nisbet, 1990), while Mathieu *et al*. (1996) reported no effects. These conflicting results on CH_4 production are likely due to strain difference of *S. cerevisiae* and type of diets (Patra, 2012). Supplementing diets with *S. cerevisiae* were shown to increase total volatile fatty acids (VFA) and propionic acid production (Mao *et al*., 2013). *S. cerevisiae* can enhance fungal colonisation of plant cell walls leading for increased DM and NDF digestion (Patra, 2012), increased initial rate of fibre digestion (Williams *et al*., 1991), improved *in situ* crude protein (CP) and NDF degradation.

Many methods can be used to *S. cerevisiae* administration. Most products contain a mixture of varying proportions of live and dead *S. cerevisiae* cells. Those with a predominance of live cells are sold as live yeasts, while others containing more dead cells and the growth medium are sold as *S. cerevisiae*. Hence, the method of *S. cerevisiae* administration may affect the potential response. The response depends on number of live or metabolically active yeast cells have shown to have a greater effect in stimulating rumen fermentation (Dawson *et al*., 1990). Direct-fed microbial are often recommended in various European countries than other administration methods (Doreau and Jouany, 1998).

The aim of this study was to determine effects of increasing doses of the yeast (*Saccharomyces cerevisiae*) in two methods of applications (direct or 72 h of pre-incubation) on *in vitro* GP, degradability and some ruminal fermentation parameters of the fibrous feedstuffs of corn stover, oat straw, sugarcane bagasse, and sorghum straw.

Materials and methods

Fibrous feed species and yeast product levels

Three individual samples of each of the fibrous feeds corn stover, oat straw, sugarcane bagasse, and sorghum straw were randomly and manually harvested in triplicate from different sites in the State of Mexico. Samples were dried at 60°C for 48 h in a forced air oven

to constant weight, ground in a Wiley mill to pass a 1 mm sieve and stored in plastic bags for subsequent determination of chemical components and *in vitro* GP. Four levels of commercial yeast product (*Saccharomyces cerevisiae* I-1077, LEVUCELL® SC20; Lalleland, Montréal, QC, Canada) contain 1×10^{10} per gram yeast product. Doses of yeast were (/g DM): control (0 mg), low (4 mg), medium (8 mg) and high (12 mg). Feed samples were incubated with yeast doses that were added into the bottles immediately before incubation (direct method) or 72 h pre-incubated at room temperature. Stock solution of each yeast product doses was prepared before treatments in distilled water in order to get the suitable doses in each 1 mL of the stock solution.

In vitro incubations

Rumen inoculum was collected from two Brown Swiss cows (400 to 450 kg body weight) fitted with permanent rumen cannula. Cows were fed *ad libitum* a total mixed ration made up of 50:50 commercial concentrate (PURINA®, St. Louis, MO, USA) containing (g/kg) 147.3 CP, 160.4 NDF, 27.7 acid detergent fibre (ADF). Alfalfa hay was formulated to meet all of their nutrient requirements (National Research Council, 2001). Fresh water was available to cows at all times during the rumen inoculum collection phase.

Ruminal contents from each cow was obtained before the morning feeding, flushed with CO_2, then mixed and strained through four layers of cheesecloth into a flask with O_2 free headspace. Samples (1 g) of each feed were weighed into 120 mL serum bottles with appropriate addition of *S. cerevisiae* doses/g DM. Consequently, 10 mL of particle free ruminal fluid was added to each bottle followed by 40 mL of the buffer solution according to Goering and Van Soest (1970), with no trypticase added, in a 1:4 (v/v) proportion.

During incubations, it was used 4 feedstuffs of 3 individual samples of each with the 4 doses of *S. cerevisiae* in 2 application methods (direct addition or 72 h pre-treatments) of *S. cerevisiae* and 4 bottles (replicates) were used for each incubated sample during 3 runs of incubation. Once all bottles were filled, they were immediately closed with rubber stoppers, shaken and placed in the incubator at 39°C. The volume of gas produced was recorded at times of 2, 4, 6, 8, 10, 12, 14, 24, 30, 48, 54 and 72 h of incubation using the pressure reading technique (Extech instruments, Waltham, MA, USA) of Theodorou *et al.* (1994). At the end of incubation (*i.e.* 72 h), bottles were uncapped, pH was measured using a pH meter (Conductronic pH15; Conductronic, Puebla, Mexico) and the contents of each bottle were filtered to obtain the non-fermented residue for determination of degraded substrate. After recording the final gas volume (*i.e.*, 72 h), 2 mL of NaOH (10 M) were added to each bottles and gas pressure was determined immediately. Mixing of the contents with NaOH allowed absorption of CO_2, with the gas volume remaining in the head space of bottles considered to be CH_4 (Demeyer *et al.*, 1988).

Degradability and sample analysis

At the end of incubation (*i.e.* 72 h), the contents of each serum bottle were filtered under vacuum through glass crucibles with a sintered filter (coarse porosity no. 1, pore size 100 to 160 µm; Pyrex, Stone, UK). Fermentation residues were dried at 105°C overnight to estimate DM disappearance with loss in weight after drying being the measure of undegradable DM. The NDF and ADF were calculated in the residues after DM degradability (DMD) determinations for establishing NDF and ADF degradability. Neutral detergent fibre was assayed without use of an alpha amylase but with sodium sulfite in the NDF. Both NDF and ADF are expressed without residual ash. Neutral detergent fibre and ADF were also determined in the residues samples after incubations for NDF and ADF degradability. Samples of the feeds were analysed for DM (#934.01), ash (#942.05), nitrogen (#954.01) and ether extract (#920.39) according to AOAC (1997). The NDF (Van Soest *et al.*, 1991), ADF, and lignin (AOAC, 1997; #973.18) analyses used an ANKOM200 Fibre Analyser Unit (ANKOM Technology Corp., Macedon, NY, USA).

Calculations and statistical analyses

All the calculations were mentioned and descripted before in Salem (2012) as in the following.

Kinetic parameters of GP were estimated (mL/g DM) by fitted data in the NLIN option of SAS (2002) according to France *et al.* (2000) as:

$$A=b\times(1-e^{-c(t-L)})$$

where A is the volume of GP at time t; *b* is the asymptotic GP (mL/g DM); *c* is the rate of GP (/h), and *L* (h) is the discrete lag time prior to gas production.

Metabolisable energy (ME; MJ/kg DM) and *in vitro* organic matter digestibility (OMD; g/kg OM) were estimated according to Menke *et al.* (1979) as:

ME=2.20+0.136 GP (mL/0.5 g DM)+0.057 CP (g/kg DM) OMD=148.8+8.89 GP+4.5 CP (g/kg DM)+0.651 ash (g/kg DM)

where GP is net GP in mL from 200 mg of dry sample after 24 h of incubation.

The partitioning factor at 24 h of incubation (PF_{24}; a measure of fermentation efficiency) was calculated as the ratio of DMD *in vitro* (mg) to the volume (mL) of GP at 72 h (*i.e.*, DMD/total gas production (GP_{96}) according to Blümmel *et al.* (1997). Gas yield (GY_{24}) was calculated as the volume of gas (mL gas/g DM) produced after 24 h of incubation divided by the amount of DMD (g) as:

Gas yield (GY_{24})=mL gas/g DM/g DMD

Short chain fatty acid (SCFA) concentrations were calculated according to Getachew *et al.* (2002) as:

SCFA (mmol/200 mg DM)=0.0222 GP-0.00425

where GP is the 24 h net gas production (mL/200 mg DM).

The experimental design for the *in vitro* ruminal GP, degradability and fermentation parameters analysis was a completely random design considering, as fixed factors, type of forage (S) and *S. cerevisiae* level (C) in the linear model (Steel *et al.*, 1997) within each method (M) of application (direct or pre-incubation). Data of each of the three runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample within each species (three samples of each) were used as the experimental unit. The statistical model was:

$$Y_{ijklm}=\mu+S_j+C_k+M_l+(S\times C)_{jk}+(S\times M)_{jl}+(M\times C)_{lk}+(S\times M\times C)_{jkl}+E_{ijklm}$$

where Y_{ijk}=is every observation of the i^{th} fibrous species (S_i) when incubated in the j^{th} yeast (C_j; *S. cerevisiae*); µ is the general mean; S_i (i=1-4) is the feed effect; C_j is the yeast dose effect (j=1-4); M_j is the application method (j=1-2), $(S^*C)_{ij}$ is the interaction between feed and yeast dose; $(S^*M)_{jl}$ is the interaction between feed and application methods; $(S^*M^*C)_{jkl}$ is the interaction between the three variable study (feed, yeast and application method); and E_{ijklm} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses of feeds to increasing addition levels of the *S. cerevisiae*.

Results

The chemical composition varied between

different species. Corn stover had higher (P<0.05) CP content with low (P<0.05) content of ADF and NDF fractions. Oat and sorghum straws had the highest (P<0.05) ADF and NDF fractions contents with low (P<0.05) content of CP (Table 1).

Fibrous species×method of application×*S. cerevisiae* interaction occurred (P<0.0001) for *b*, *c*, *L*, GP, CH_4 production, ME, SCFA, GY_{24} and PF_{24} (Tables 2 and 3).

Effect of fibrous species

Gas production parameters were varied (P<0.001) between different fibrous species. Corn stover and oat straw improved the asymptotic gas production with increasing of the initial delay before GP beginning followed by sorghum straw and sugarcane baggas which had the rate of GP increased. During the first 14 h of incubation, sugarcane baggas improved (P<0.001) *in vitro* GP followed by corn stover where oat straw had the lowest (P<0.001). After 14 h of incubation, corn stover and sugarcane bagass improved (P<0.001) *in vitro* GP followed by oat straw, while sorghum straw had the lowest (P<0.001). After 24 h of incubation only corn stover showed a gas production higher (P<0.001) than oat and sorghum straw (P<0.001), while sugarcane baggas did not improve gas production compared to the straws (Table 2). Oat straw, sugarcane baggas, and sorghum straw had both ruminal pH (P=0.006) and GY_{24} (P<0.001) increased with lowering (P<0.001) PF_{24} value compared to corn stover. Corn stover had DMD, OMD, ME, SCFA, and PF_{24} values improved (P<0.001). On the contrary, sorghum straw had the highest (P<0.001) values of CH_4, NDFD, and ADFD compared to the other feeds. Sugarcane baggas had the lowest values (P<0.001) of CH_4, NDFD, OMD, and ME, where oat straw had the lowest (P<0.001) SCFA production compared to other feeds (Table 3).

Effect of application methods

Direct addition of *S. cerevisiae* improved (P<0.001) the rate of GP with lowering (P<0.001) the initial delay before GP beginning. The *in vitro* GP was also improved (P<0.001) compared to the 72 h pre-incubation method (Table 2).

The direct addition of *S. cerevisiae* also improved CH_4 (P=0.047), DMD (P=0.005), NDFD (P=0.005), ADFD (P=0.020), (P<0.001)

Table 1. Chemical composition of the four fibrous feeds.

Species	OM, g/kg DM	CP, g/kg DM	ADF, g/kg DM	NDF, g/kg DM
Corn stover	959.7	62.9^a	274.4^c	476.7^b
Oat straw	923.6	37.2^b	380.0^a	537.8^a
Sugarcane bagasse	982.0	25.7^c	324.4^b	458.9^b
Sorghum straw	944.3	40.0^b	377.8^a	556.7^a
SEM	42.3	9.3	28.1	37.1

OM, organic matter; DM, dry matter; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre. $^{a-c}$Different superscripts following means in the same row indicate differences at P<0.05.

Table 2. *In vitro* rumen gas kinetics of four low quality roughages as affected by the direct addition or 72 h pre-incubation with different levels of *Saccharomyces cerevisiae*.

	GP parameters			*In vitro* GP, mL/g DM										
	b, mL/g DM	c, /h	L, /h	Gas2	Gas4	Gas6	Gas8	Gas10	Gas12	Gas14	Gas24	Gas30	Gas48	Gas54	Gas72
Effect of S															
Corn stover	329.5^a	0.029^c	0.74^b	18.2^b	35.4^b	51.6^b	66.9^b	81.3^b	94.9^b	107.8^b	162.0^a	187.9^a	243.4^a	256.5^a	284.8^a
Oat straw	273.9^b	0.029^c	1.14^a	14.5^c	28.2^c	41.0^c	53.1^d	64.5^d	75.2^d	85.4^d	128.1^c	148.7^b	193.8^b	204.7^b	229.1^b
Sugarcane baggas	159.0^d	0.099^a	0.36^c	28.4^a	51.1^a	69.4^a	84.2^a	96.3^a	106.2^a	114.4^a	138.8^b	146.1^b	155.4^c	156.6^c	158.3^c
Sorghum straw	257.4^c	0.041^b	0.45^c	17.5^b	33.4^b	48.1^b	61.6^c	74.0^c	85.4^c	96.0^c	138.1^b	156.9^c	195.4^b	204.2^b	223.2^b
LSD	15.27	0.0064	0.153	1.56	2.71	3.54	4.16	4.62	4.97	5.25	6.11	6.54	8.02	8.53	9.99
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Effect of application M															
Direct	256.1	0.056^a	0.51^b	22.3^a	41.6^a	58.4^a	73.3^a	86.6^a	98.4^a	109.2^a	150.3^a	168.1^a	203.8^a	211.8^a	228.8^a
Pre- incubation	253.8	0.043^b	0.84^a	17.0^b	32.5^b	46.7^b	59.6^b	71.5^b	82.5^b	92.6^b	133.2^b	151.7^b	190.2^b	199.2^b	218.8^b
LSD	8.19	0.0034	0.082	0.84	1.45	1.90	2.23	2.48	2.67	2.82	3.28	3.51	4.30	4.58	5.36
P	0.5895	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Effect of Y, mg/g DM															
0	243.1^b	0.041^b	0.55^b	16.5^c	31.7^c	45.7^c	58.5^c	70.3^c	81.2^c	91.2^c	131.5^c	149.6^c	186.3^c	194.7^c	212.6^c
4	259.4^a	0.045^b	0.66^{ab}	19.0^b	36.1^b	51.6^b	65.8^b	78.6^b	90.4^b	101.2^b	143.7^{ab}	162.4^{ab}	200.4^{ab}	209.0^{ab}	227.5^{ab}
8	251.9^{ab}	0.056^a	0.74^a	21.0^a	39.2^a	55.1^{ab}	69.2^{ab}	81.6^{ab}	92.8^{ab}	103.0^{ab}	142.1^b	159.3^b	194.8^b	203.1^{bc}	221.1^{bc}
12	265.4^a	0.056^a	0.75^a	22.1^a	41.1^a	57.7^a	72.4^a	85.6^a	97.4^a	108.1^a	149.8^a	168.3^a	206.4^a	215.2^a	234.1^a
LSD	15.27	0.0064	0.153	1.56	2.71	3.54	4.16	4.62	4.97	5.25	6.11	6.54	8.02	8.53	9.99
Linear	0.0065	0.1165	0.076	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002
Quadratic	0.006	<0.0001	0.0066	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interactions															
S×M	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0024
S×Y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
M×Y	0.0989	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S×M×Y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0002

GP, gas production; DM, dry matter; b, asymptotic gas production; c, rate of gas production; L, initial delay before gas production begins; LSD, least significant difference; S, species; M, method; Y, yeast product. $^{a-d}$Different superscripts following means in the same row indicate differences at P<0.05.

OMD, SCFA, and GY_{24}, with lowering ($P<0.001$) PF_{24} compared to the 72 h pre-incubation method. The method of application did not affect ($P>0.05$) both of the asymptotic gas production and ruminal pH (Table 3).

Effect of yeast doses

Both low and high doses of *S. cerevisiae* improved the asymptotic GP, with increasing *S. cerevisiae* doses, the rate of GP (linear, P=0.007; quadratic, P=0.006), the initial delay before GP beginning (quadratic, $P<0.001$), and *in vitro* GP (linear, quadratic, $P\leq0.001$) during the period before the first 24 h. After 24 h and up to 72 h of incubation, the highest dose of *S. cerevisiae* had the *in vitro* GP increased (linear, quadratic, $P<0.001$) where the lowest dose had the lowest *in vitro* GP compared to other doses (Table 2).

The lowest dose of *S. cerevisiae* improved DMD (linear, P=0.005; quadratic, $P<0.001$), and PF_{24} (linear, $P<0.001$; quadratic, P=0.011) with lowering (linear, quadratic, $P<0.001$) ME, OMD, SCFA, and (linear, $P<0.001$; quadratic, P=0.005) GY_{24} compared to the other doses. In contrary, the highest dose of *S. cerevisiae* had the highest values of DMD, ME, OMD, and SCFA (Table 3). No effects ($P>0.05$) of *S. cerevisiae* doses on ruminal pH, CH_4, and ADFD; however, addition of yeast caused a lowered (linear, P=0.005) values for NDFD (Table 3).

Discussion

Chemical composition

The chemical composition varied between the four fibrous feeds used in our study. These variations arise from variation in the genotype of the crops, differences between production environments, and from the interaction between environment and genotypes (Welch, 1995; Denčić *et al*., 2011). Environmental differences will include variation in climate, the soil and agronomic practice, together with variations raised from different harvesting conditions, and post harvesting treatments (Welch, 1995; Elghandour *et al*. 2013). There is usually an inverse relationship between the CP and crude fibre content in a given forage species, and this has been revalidated in this study.

Gas production, rumen fermentation and degradability

The responses to *S. cerevisiae* are fibrous species type, forage composition, application methods and dose-dependent in addition to interactions among yeast and diet (Patra, 2012). Gas production from different fibrous species depends on its chemical composition. In our study, during the first 24 h of incubation, sugarcane bagasse and corn stover produced more GP than sorghum and oat straw compared to the period after 24 h up to 72 where they produced more gases. The production of gases from tested roughages depends on portentous and fibrous contents of feeds (Paya *et al*., 2007). Higher GP during the first period of fermentation of both sugarcane bagasse and corn stover refers to high content of highly fermentable constituents than sorghum and oat straw. Conversely, the fermentation process of sorghum and oat straw refers to their content of low fermentable constituents. Gas production depends on nutrient availability for rumen microorganisms (Mahala and Fadel Elseed, 2007). Fermenta-

Table 3. *In vitro* rumen fermentation profile of four low quality roughages as affected by the direct addition or 72 h pre-incubation with different levels of *Saccharomyces cerevisiae*.

	pH	CH_4, mL/g DM	DMD, mg/g DM	NDFD, mg/g DM	ADFD mg/g DM	ME, MJ/kg DM	OMD, g/kg DM	SCFA, mmol/g DM	PF_{24}, mg DMD:mL gas	GY_{24}, mL gas/g DMD
Effect of S										
Corn stover	6.78^{b}	18.7^{b}	326.1^{a}	256.7^{c}	264.6^{c}	6.84^{a}	459^{a}	3.58^{a}	2.03^{a}	497.4^{b}
Oat straw	6.98^{a}	17.1^{b}	285.0^{b}	276.6^{b}	363.0^{a}	6.12^{bc}	413^{bc}	2.82^{c}	2.24^{b}	450.1^{a}
Sugarcane baggas	6.97^{a}	6.8^{c}	272.4^{c}	217.2^{d}	310.1^{b}	6.08^{c}	405^{c}	3.06^{b}	2.00^{b}	511.4^{a}
Sorghum straw	7.00^{a}	25.2^{a}	270.9^{c}	291.1^{a}	360.7^{a}	6.27^{b}	422^{b}	3.04^{b}	1.99^{b}	517.1^{a}
LSD	0.153	2.12	4.69	7.00	4.78	0.166	10.9	0.136	0.117	24.42
P	0.0006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Effect of application M										
Direct	6.91	17.5^{a}	290.4^{a}	263.2^{a}	326.1	6.56^{a}	440^{a}	3.32^{a}	1.96^{b}	522.1^{a}
Pre-incubation	6.96	16.4^{b}	286.7^{b}	257.7^{b}	323.1	6.09^{b}	410^{b}	2.94^{b}	2.18^{a}	465.9^{b}
SEM	0.082	1.14	2.52	3.75	2.60	0.089	5.83	0.073	0.063	13.11
P	0.267	0.0467	0.0046	0.0045	0.0204	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Effect of Y product, mg/g DM										
0	6.92	17.6	290.4^{a}	263.3^{a}	325.4	6.05^{c}	407^{c}	2.90^{c}	2.23^{a}	454.5^{b}
4	6.97	17.0	283.9^{b}	255.4^{b}	325.3	6.38^{ab}	428^{ab}	3.17^{ab}	2.00^{b}	513.9^{a}
8	6.92	17.0	285.3^{b}	261.4^{ab}	323.7	6.34^{b}	426^{b}	3.13^{b}	2.03^{b}	500.0^{a}
12	6.93	16.3	294.8^{a}	261.6^{ab}	324.0	6.55^{a}	439^{a}	3.30^{a}	2.01^{b}	507.7^{a}
LSD	0.153	2.12	4.69	6.70	4.78	0.166	10.9	0.136	0.117	24.42
Linear	0.3417	0.4311	0.0005	0.0045	0.9362	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Quadratic	0.7076	0.1493	<0.0001	0.3303	0.3932	<0.0001	<0.0001	<0.0001	0.011	0.0046
Interactions										
S×M	0.1869	0.2509	<0.0001	<0.0001	0.6104	<0.0001	<0.0001	<0.0001	0.0011	<0.0001
S×Y	0.8837	<0.0001	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
M×Y	0.8066	0.4184	0.1636	0.0001	0.4479	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S×M×Y	0.9481	<0.0001	<0.0001	<0.0001	0.0152	<0.0001	<0.0001	<0.0001	0.0032	<0.0001

CH_4, methane emission; DM, dry matter; DMD, dry matter degraded substrate; NDFD, neutral detergent fibre degradability; ADFD, acid detergent fibre; ME, metabolisable energy; OMD, organic matter digestibility; SCFA, short chain fatty acids; PF24, partitioning factor at 24 h of incubation; GY_{24}, gas yield at 24 h; S, species; LSD, least significant difference; M, method; Y, yeast product. $^{a-d}$Different superscripts following means in the same row indicate differences at $P<0.05$.

tion of dietary carbohydrates to acetate, propionate and butyrate produces gases in the rumen which are mainly composed of hydrogen, carbon dioxide and methane. However, fermentability of protein produces relatively small GP compared to carbohydrate fermentation (Makkar *et al*., 1995). This can explain how *S. cerevisiae* addition could improve GP at the time it reduced NDFD. All depends on the chemical composition of fermented feeds. Availability of nutrients for rumen microorganisms will stimulate the degradability of different nutrients (Paya *et al*., 2007). It is very important to stress that not only has the addition of *S. cerevisiae* the ability to improve GP, but it can also make qualitative changes in GP thereby reducing its negative effect on the environment. *S. cerevisiae* has the ability to decrease methane and ammonia production, and to improve fermentation efficiency to contribute to a reduction in greenhouse gas emissions (Hristov *et al*., 2013). Moreover, decreasing protein degradation and ammonia production in the rumen (Mao *et al*., 2013) has the ability to decrease the overall nitrogen excretion by the animal, which would contribute to decreased ammonia emissions from cattle manure.

There are a few data in the literature regarding the effect of *S. cerevisiae* method of application on *in vitro* gas kinetics and fermentation profile. It has been found that the method of the *S. cerevisiae* product application depends on number of live or metabolically active *S. cerevisiae* that will stimulate rumen fermentation (Dawson *et al*., 1990). Direct application of *S. cerevisiae* ensures the viability of *S. cerevisiae* cells so an improvement occur for *in vitro* GP and fermentation kinetics and profile compared with pre-incubation method. Pre-incubation of *S. cerevisiae* with different fibrous feeds may negatively affect the fermentation process which reflected on low fermentability for all fibrous species. Doreau and Jouany (1998) stated that direct-fed microbial are often recommended in various European countries than other administration methods. Elam *et al*. (2003) hypothesised that the initial advantages of direct-fed microbial involve a favourable alteration of the gastrointestinal micro-flora and that over time that innate immunological mechanisms of control animals provide this same function.

Improved GP with increasing *S. cerevisiae* doses reflects the enhanced ruminal environment. Paulus *et al*. (2012) and Mao *et al*. (2013) documented the positive effects of *S. cerevisiae* on ruminal fermentation and microbial activities. A number of specific hypothetical biochemical mechanisms have been developed to explain the stimulatory effects of *S. cerevisiae* in the rumen (Chevaux and Fabre, 2007). Some of these mechanisms have been based on the ability of yeast to provide important nutrients or nutritional cofactors that stimulate microbial activities (Callaway and Martin, 1997). Another suggested the ability of *S. cerevisiae* to scavenge excess oxygen creating a more optimal environment for rumen anaerobic bacteria (Newbold *et al*., 1996; Jouany, 2001). Others studies suggested that *S. cerevisiae* supplementation could provide vitamins such as biotin and thiamine, which are reported to be required for microbial growth and activity (Akin and Borneman, 1990). In addition, others suggested that *S. cerevisiae* can provide a focal point for the development of a stable microbial consortium (Jouany, 2001). In this model, the *S. cerevisiae* cells provide a site for metabolic exchanges and an environment that promotes the growth of beneficial microorganisms around substrates.

One possible explanation for the varied response with a different level of *S. cerevisiae* in this study is at least partially due to the nature of the *in vitro* procedure. For the *in vitro* model, the substrate amount relative to the rumen liquid volume is much less than in the rumen of a cow (<1 *vs* 12%). Therefore, when a rumen modulator like *S. cerevisiae* is supplemented at a different rate, it could change the fermentation rate and cause different substrate depletion, resulting in different response as the fermentation length is changed (Mao *et al*., 2013). Lila *et al*. (2004) found variable effects of *S. cerevisiae* on ruminal fermentation when different substrates were used *in vitro*.

Decreased lag time with *S. cerevisiae* addition can be illustrated based on two basic mechanisms. The first mode of yeast action reported by Newbold *et al*. (1996) is the respiratory activity that scavenges O_2, which is toxic to anaerobic bacteria and causes inhibition of adhesion of cellulolytic bacteria to cellulose, and this peak in O_2 concentration occurs at approximately the time of feeding (initial time). The second mode is that *S. cerevisiae* contains small peptides and other nutrients that required to predominant ruminal cellulolytic bacteria to initiate growth (Callaway and Martin, 1997).

Addition of *S. cerevisiae* increased SCFA production on forage substrates (Mao *et al*., 2013). Increased SCFA production and ME are associated with high activities of microbes in the rumen. *S. cerevisiae* produces growth factors for microbial growth that can stimulate rumen microbial growth and activity (Chiquette, 2009). In addition to the ability of *S. cerevisiae* to provide conducive conditions to microbial growth in a way that is capable of using O_2 in the rumen so that the conditions of an aerobic rumen awake (Mosoni *et al*., 2007). *S. cerevisiae*. Newbold *et al*. (1996), for example, used this mode of action to explain a 35% increase in total bacterial counts with *S. cerevisiae in vitro*.

Addition of *S. cerevisiae* lowered PF_{24} values. A lower PF_{24} would reflect lower conversion of degraded substrate into microbial biomass and vice versa (Harikrishna *et al*., 2012). Ruminal pH was not changed during fermentation processes. Several studies have suggested that *S. cerevisiae* moderate the ruminal pH by increasing lactate utilisation making pH relatively more stable and meet the needs of rumen microbes to perform its activity (Paulus *et al*., 2012).

Conclusions

The responses to supplemental *S. cerevisiae* varied among the fibrous species tested, the results of this study suggest that the addition of *S. cerevisiae* can support ruminal fermentation of low-quality forages. In general, *S. cerevisiae* added at 4 to 12 g/kg DM showed the greatest responses in most variables tested.

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In vitro gas and methane production of two mixed rations influenced by three different products of *Saccharomyces cerevisiae*

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Abstract

The current study aimed to evaluate if the effect of *Saccharomyces cerevisiae* (SC) on *in vitro* fermentation can be affected with the crude protein (CP) content of the ration. Three commercial *Saccharomyces cerevisiae* (SC) cultures of Biocell F53®, Procreatin 7® and Biosaf SC47® were evaluated at 0 (SC0), 2 (SC2), and 4 (SC4) mg/g DM of substrate. Two rations with 13% (LCP) and 16% CP (HCP) were used as substrates. Rumen gas (GP) and methane (CH_4) productions were recorded at 2, 4, 6, 8, 10, 12, 14, 24, and 48 h of incubation. Interactions were observed (P<0.05) between ration × yeast culture × yeast dose for GP, CH_4 production, and fermentation kinetic parameters. The HCP ration had increased (P=0.05) asymptotic GP, CH_4 production, and fermentation parameters. Biocell F53® and Biosaf SC47® increased the asymptotic GP (P<0.05) in HCP and LCP rations with better effect for the dose of 2 mg/g DM substrate HCP (P<0.05) and dose of 4 mg yeast/g DM substrate

with the LCP ration. The highest CH_4 production was observed (P<0.05) with Procreatin 7®. Moreover, Procreatin 7® at 2 mg/g DM had improved (P<0.05) fermentation kinetics of the HCP ration than other doses of other yeast cultures, while with the LCP ration, the dose of 2 mg/g DM from the Biocell F53® had better fermentation kinetics (P<0.05). It could be concluded that HCP ration improved GP than LCP ration. Moreover, addition of Biocell F53® and Biosaf SC47® at rate of 2 mg/g DM improved fermentation kinetics and nutrients degradability.

Key words: degradability, methane, protein level, yeast.

Abbreviations:

ADF, acid detergent fiber; *b*, the asymptotic gas production; *c*, the rate of gas production; CH_4, methane; CP, crude protein; DM, dry matter; DMD, DM degradability; GP, gas production; GY24, gas yield at 24 h of incubation; HCP, high crude protein; *L*, the initial delay before gas production begins*;* LCP, low crude protein; MCP, microbial CP production; ME, metabolizable energy; NDF, neutral detergent fiber; OM, organic matter; OMD, *in vitro* OM digestibility; PF_{24}, partitioning factor at 24 h of incubation; SC, *Saccharomyces cerevisiae*.

1. Introduction

One of the most important problems facing ruminant production is the losing of energy and high biological value proteins as a result of ruminal fermentation. This may cause a limited productive performance (Salem et al.
2014a; Kholif et al. 2014) and release of pollutants to the environment (Calsamiglia et al. 2007). Ionophores and antibiotics have good results to reduce these losses in energy and protein (McGuffey et al. 2001); however, the European Union banned the use of them due to the potential of appearance of residues in milk or meat (Russell and Houlihan 2003). Nowadays, researches are concerning the use of natural feed additives, generally recognized as safe for human consumption, including phytogenic extracts (Valdes et al. 2015),

enzymes (Alsersy et al. 2015) or *Saccharomyces cerevisiae* (SC) (Elghandour et al. 2015a) to modify rumen microbial fermentation.

The SC is generally recognized as safe by the US Food and Drug Administration, and they can be legally used as animal feed additives. Yeast as a natural feed additive, has the ability to stabilize rumen fermentation and prevents rumen flora disorders and disturbances (Pinloche et al. 2013) with increasing the numbers of viable bacterial cells (Jouany 2001). Enhanced ammonia utilization by ruminal microorganisms is another benefit from using yeast (Chaucheyras-Durand et al. 2008). Moreover, SC can provide the rumen with important nutrients and nutritional cofactors in addition to vitamins, which reported to be required for microbial growth and activity (Mao et al. 2013; Polyorach et al. 2014). The SC have the ability to increase dry matter (DM) and neutral detergent fiber (NDF) digestion (Elghandour et al. 2014; 2015a), and increase initial rates of fiber digestion (Williams et al. 1991). In addition, it could enhance fungal colonization of plant cell walls resulting in increased DM and NDF digestion (Patra 2012), and improved *in situ* crude protein (CP) and NDF degradation. Elghandour et al. (2014) reported an increased *in vitro* rumen degradability of forages which was associated with ability of yeast to stimulate growth and activity of fibrolytic bacteria (Wambui et al. 2010).

Increased gas production (GP) was paralleled with administration of SC (Elghandour et al. 2014) which might stimulated the acetogens to compete or co-metabolize hydrogen with methanogens, thereby reduce methane (CH_4) emissions (Hristov et al. 2013). However, others reported increased CH_4 emission (Martin and Nisbet 1992), or not affected (Mathieu et al. 1996) with SC administration. These conflicting results on CH_4 emission are likely due to strain difference of SC and type of diets (Patra 2012). In general, there is inconsistence between reports regarding the effect of yeast on animals' performance. Some of the possible causes for the inconsistency could be associated with characteristics of the strain (Newbold et al. 1996), differences between commercial additives (Mendoza et al. 1995), and diet composition (Elghandour et al. 2014).

The commercial available SC cultures, in general, contains mixtures of varying proportions of live and dead cells. So, as expected, the response to different SC cultures will be vary depending on number of live or metabolically active SC cells, the dose used, the feeds, and/or other nutrients compounds in the cultures such as fats, proteins, ash and carbohydrates (Elghandour et al. 2014). Therefore, the current study aimed to study the effect of three SC cultures, abundant in Mexico, at different doses on GP and fermentation kinetics of two total mixed rations with high (16% CP; HCP) and low (13% CP; LCP) CP levels.

2. Materials and Methods

2.1. Substrates and yeast levels

Two mixed rations with two different levels of CP of 13% (LCP) and 16% (HCP) on DM basis (Table 1) were used as substrates to be incubated with three doses of different SC cultures.

Three types of SC cultures were tested at three doses (mg/g DM of substrate): 0 (without; SC0), 2 (SC2), and 4 (SC4). Stock solution of each yeast culture doses was prepared before treatments in distilled water in order to get the suitable doses in each 1 ml of the stock solution.

The three cultures of SC (Lesaffre Feed Additives, Toluca, Mexico) were used: (1) Biocell® contains a minimum guarantee of 2.0×10^{10} CFU/g *Saccharomyces cerevisiae*. (2) Procreatin 7® contains minimum guarantee of 1.5×10^{10} CFU/g *Saccharomyces cerevisiae*. (3) Biosaf SC47® contains, as a minimum count of live yeast cell 1.0×10^{10} CFU/g *Saccharomyces cerevisiae*.

2.2. In vitro incubations

As described before in Elghandour et al. (2014), three sheep (35 to 45 kg body weight) fitted with permanent rumen cannula were used as rumen inoculum donors and fed on a total mixed ration of commercial concentrate and corn silage at 1:1 DM formulated to cover their nutrient requirements (NRC 1985). Sheep had a free access to fresh water during all times of rumen inoculum collection phase.

Before the morning feeding, ruminal contents were obtained from each sheep and flushed with CO_2 to keep it anaerobically, then mixed and strained through four layers of cheesecloth into a flask with O_2 free headspace. Feeds samples (0.5 g) were weighed into 120 ml serum bottles with appropriate addition of yeast cultures doses/g DM. Ten ml of particle free ruminal fluid were added to each bottle followed by 40 ml of the buffer solution according to Goering and Van Soest (1970), with no trypticase added, in a 1:4 (vol/vol) proportion.

Once all bottles were filled, they were immediately closed with rubber stoppers, shaken and placed in the incubator at 39 ºC. The volume of gas produced and CH_4 production were recorded at times of 2, 4, 6, 8, 10, 12, 14, 24, and 48 h of incubation. Gas production was recorded using the pressure reading technique (Extech instruments, Waltham, USA) of Theodorou et al. (1994) while the methane emission was recorded using Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK).

After 48 h of incubation, bottles were uncapped, pH was measured using a pH meter and the contents of each bottle were filtered to obtain the non- fermented residue for determination of degraded substrate.

2.3. Degradability and sample analysis

Degradability and analysis were determined as it was descripted in Elghandour et al. (2014). Briefly, after 48 h of incubation, the fermentation process was stopped where the contents of each serum bottle were filtered under vacuum through glass crucibles with a sintered filter. The obtained fermentation residues were dried at 105 ºC overnight to estimate DM disappearance. Both of NDF and acid detergent fiber (ADF) were determined in the residues after DM degradability (DMD) determinations for determining the degradability of NDF (NDFD) and ADF (ADFD). Samples of the feeds were analyzed for DM (#934.01), ash (#942.05), N (#954.01) and EE (#920.39) according to AOAC (1997). The NDF and ADF content of both feeds and fermentation residues were determined using an ANKOM200 Fibre Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA) without use of an alpha

amylase but with sodium sulfite in the NDF (Van Soest et al. 1991). Both NDF and ADF are expressed without residual ash.

2.4. Calculations and statistical analyses

As descripted before in Salem et al. (2014b), to estimate kinetic parameters of GP, results (ml/g DM) were fitted using the NLIN option of SAS (2002) according to France et al. (2000) model as:

$$A = b \times (1 - e^{-c(t-L)})$$

where: A is the volume of GP at time t; *b* is the asymptotic GP (ml/g DM); *c* is the rate of GP (/h), and *L* (h) is the discrete lag time prior to GP.

Metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter digestibility (OMD, g/kg OM) were estimated according to Menke et al. (1979) as:

ME = 2.20 + 0.136 GP (ml/0.5 g DM) + 0.057 CP (g/kg DM)

OMD = 148.8 + 8.89 GP + 4.5 CP (g/kg DM) + 0.651 ash (g/kg DM)

where: GP is net GP in ml from 200 mg of dry sample after 24 h of incubation.

The partitioning factor at 24 h of incubation (PF_{24}; a measure of fermentation efficiency) was calculated as the ratio of DM degradability in vitro (DMD, mg) to the volume (ml) of GP at 24 h (i.e., DMD/total GP (GP_{24})) according to Blümmel et al. (1997). Gas yield (GY_{24}) was calculated as the volume of gas (ml gas/g DM) produced after 24 h of incubation divided by the amount of DMD (g) as:

Gas yield (GY_{24}) = ml gas/g DM/g DMD

The experimental design for the *in vitro* ruminal GP, CH_4 emission, degradability and fermentation parameters analysis was a completely random design considering, as fixed factors, type of ration (R) and yeast culture doses (D) in the linear model (Steel et al. 1997) within each yeast culture (P). Data of each of the three runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample within each species (three samples of each) were used as the experimental unit. The statistical model was:

$$Y_{ijkl}=\mu+ R_i+D_j+P_k+(R*D)_{ij}+(R*P)_{ik}+(P*D)_{jk}+(R*P*D)_{ijk}+E_{ijkl}$$

where: Y_{ijkl} = is every observation of the i^{th} ration (R_i) when incubated in the j^{th} level D_j; μ is the general mean; R_i (i=1-2) is the ration effect; D_j is the yeast doses effect (j=1-3); P_k is the culture type (j= 1-3), $(R*D)_{ij}$ is the interaction between ration and yeast doses; $(R*P)_{jk}$ is the interaction between ration and culture; $(R*P*D)_{ijk}$ is the interaction between rations, yeast cultures and doses; and E_{ijkl} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses of feeds to increasing addition doses of the yeast cultures.

3. Results

3.1. Gas and methane productions

Interactions were observed (P < 0.05) between ration × yeast culture, yeast culture × yeast dose, and between ration × yeast culture × yeast dose for gas and methane productions. Compared to the LCP and without yeast addition (control treatments), HCP had increased (P = 0.001) asymptotic gas production and decreased lag time (P < 0.001) without affecting the rate of GP (P > 0.05). Compared to control treatments, Biocell F53® and Biosaf SC47® increased the asymptotic GP (P < 0.001) in both rations where the dose of 2 mg yeast/g DM substrate was more effective (linear effect, P = 0.001; quadratic effect, P = 0.023) than the dose of 4 mg yeast/g DM substrate with the HCP ration. Regarding the LCP ration, the dose of 4 mg yeast/g DM substrate was more effective (linear effect, P = 0.001; quadratic effect, P = 0.023) to increase the asymptotic gas production in both Biocell F53® and Procreatin 7® than the low dose (Table 2).

Increased methane production was observed (P < 0.001) with the LCP ration than HCP ration after 24 and 48 h of incubation. For the HCP ration, the highest CH4 productions at 24 h of incubation were observed (P < 0.01) with Procreatin 7® (at 2 mg/g DM) and with Biosaf SC47® (at 2, and 4 mg/g DM), while at 48 h of incubation was observed with the Procreatin 7® at 4 mg/g DM (P < 0.001). For the LCP ration, Biocell F53® and Biosaf SC47® had the highest CH_4 production at 24 h of incubation (P < 0.01), while at 48 h of incubation the dose

of 4 mg/g DM of all tested yeast cultures decreased (P <0.01) CH_4 at 48 h of incubation (Table 2).

3.3. Nutrients degradability and fermentation kinetics

Interactions between ration × yeast culture, yeast culture × yeast dose, and ration × yeast culture × yeast dose were observed (P < 0.05) for ME, PF_{24}, MCP, GY_{24}, DMD and OMD. The HCP ration had increased (P > 0.05) ME, MCP, GY_{24}, DMD and OMD with decreased PF_{24} compared to the LCP ration. Yeast culture had no effect on fermentation kinetics and nutrients degradability with exception of DMD (P < 0.001). With the HCP ration, addition of Procreatin 7® at 2 mg/g DM had increased ME, MCP, GY_{24}, DMD and OMD with decreased PF24 compared to the other doses of other yeast cultures. In the contrary and with the LCP ration, the dose of 2 mg/g DM from the culture Biocell F53® had increased ME, MCP, GY_{24}, and OMD compared to other doses of different yeast cultures; however, the dose of 2 mg/g DM of Procreatin 7® had increased DMD compared to other doses of other yeast cultures. No effect was observed (P > 0.05) on fermentation pH, NDFD and ADFD between the two rations, different yeast cultures and different yeast doses (Table 3).

4. Discussion

4.1. Gas production

Increasing protein content of the ration caused an increased GP. However, fermentability of protein produces relatively small GP compared to carbohydrate fermentation (Makkar et al. 1995). The GP, form any substrate, depends mainly on nutrient availability for rumen microorganisms (Elghandour et al. 2014; 2015b). Fermentation of dietary carbohydrates to acetate, propionate and butyrate produces gases (mainly CH_4, CO_2, H_2) in the rumen. However, in the current study, both of rations (i.e., LCP and HCP) had almost the same fiber fractions content. So, it is well clear that the increased GP was a result of increased CP content. It is well known that SC has the ability to decrease ammonia production in the rumen (Hristov et al. 2013) by decreased

protein degradation and decreased the overall N excretion by the animal, which would contribute to decreased ammonia emissions from cattle manure (Mao et al. 2013). The direct result of this action was the expected increased protein bypass in the rumen to be absorbed and metabolized as a true protein in the true stomach and small intestine.

Decreased lag time with increased protein content (i.e., HCP ration) reflects the fast activity of SC on the fermentation process. Newbold et al. (1996) stated that SC can affect the respiratory activity that scavenges O_2 (Chaucheyras-Durand et al. 2008), which is toxic to anaerobic bacteria and causes inhibition of adhesion of cellulolytic bacteria to cellulose, and this peak in O_2 concentration occurs at approximately the time of feeding (i.e., initial time). Moreover, SC contains small peptides and other nutrients that required to predominant ruminal cellulolytic bacteria to initiate growth (Callaway and Martin 1997). Activity of SC depends on many factors including availability of nutrients for rumen microorganisms will stimulate fermentation process (Paya et al. 2007). Previous studies reported that the stimulation of cellulose degradation by SC addition was associated with a decreased lag time, which results in increased initial rates of digestion, but not in increased extent of digestion by ruminal microorganisms (Williams et al. 1991).

Both of Biocell F53® and Biosaf SC47® cultures improved GP than Procreatin 7®. This may be related with the nature of each culture and their contents of live cells, and other nutrients/carrier materials.

The low dose of SC used (SC2) improved GP than the high dose (SC4). However, many reports stated an increased GP with increasing SC dose (Mao et al. 2013; Elghandour et al. 2014). The nature of substrate, and the *in vitro* procedure are responsible about the varied response with a different level of SC. In case of *in vitro* technique, the substrate amount relative to the used rumen liquid volume for incubation is much less than in the rumen of a cow (<1 *vs* 12%). In case of rumen modulator like SC supplementation at different rates, SC could change the fermentation rate and cause different substrate depletion, resulting in different responses (Mao *et al*., 2013).

4.2. CH_4 emission

Before the first 24 h of incubation, CH_4 emission was negligible and then started to be increased quickly to reach its concentration peak at the end of incubation; however, GP started early with incubation. This reflects the nature of the produced gases. During fermentation process, amounts of gases are produced within the rumen which mainly constitutes H_2, CO_2 and CH_4. As previously mentioned, increasing ration CP content caused an increased GP with decreasing CH_4 emission. This result might be due to an increased proportion of protein in the ration which changes the produced short chain fatty acids concentrations in such a way that less acetic and more propionic is formed, and hence, the supply of hydrogen for methanogenesis is limited (Polyorach et al. 2014) with reducing the protozoal population (Iqbal et al. 2008).

Methane production differed between yeast cultures. This may be illustrated based on different cultures contents from other components like CP, crude fiber, crude fat ash, and/or materials of coating.

Moreover, the low dose of SC (SC2) increased CH_4 production than the SC4. This related with the increased GP with this dose of SC and the changed nature of produced gas due to SC addition. Elghandour et al. (2014) noted an increased CH_4 production as the produced gases was increased when SC was added. However, increasing the dose of SC decreased CH_4 production. Some studies suggested that SC culture might stimulate the acetogens to compete or to co-metabolize H_2 with methanogens thereby, reducing CH_4 productions (Mwenya et al. 2004; Elghandour et al. 2014). Polyorach et al. (2014) noted that CH_4 emission in the rumen was decreased when animals fed SC fermented cassava chip protein instead of soybean meal. They returned it to the ability of SC to affect H_2 metabolism in the rumen with altering the fermentation process in a manner that reduces the formation of CH_4. However, other studies (Martin and Nisbet 1992) reported an increased CH_4 production. These conflicting

results on CH_4 production are likely due to strain difference of SC cultures and nature of rations (Patra 2012).

4.3. pH and nutrient degradabilities

Ruminal pH was not affected during fermentation processes. Several studies have suggested that SC moderate the ruminal pH by increasing lactate utilization making pH relatively more stable and meet the needs of rumen microbes to perform its activity (Elghandour et al. 2014).

Nutrients degradability showed an improved DMD and OMD without affecting on NDFD and ADFD with high protein ration (HCP) even with SC addition. However, different SC cultures affected only on DMD. Both of rations had a much closed fiber fractions contents with different CP content. So, the improved DMD and OMD were a result of increased CP which improved the microflora activity in the rumen. These could be due to increased protein level that would provide more readily available energy, enhancing corresponding of microbes due to the better supply of fermentable organic matter, energy and nitrogen to rumen bacteria, consequently, increased degradability (Polyorach et al. 2014). Bach et al. (2005) indicated that the most important factors affecting utilization of dietary protein in the rumen included type of protein, carbohydrate and their interactions and the predominant microbial population in the rumen. The unaffected NDFD and ADFD with changing protein content; however, SC was added, may be due to the high protein content of the ration. It is well known that SC had the ability to stimulate growth and activity of total ruminal anaerobes bacteria (Jouany 2001). Polyorach et al. (2014) showed that SC can increase rumen microorganism's total numbers and improve the utilization of feeds. However, most of reports showed an improved fibers fractions degradability (Elghandour et al. 2014) as a result of increased cellulolytic digester species *Fibrobacter succinogenes, Ruminococcus flavifaciens* and *Selenomonas ruminantium* (Callaway and Martin 1997). Guedes et al. (2008) stated unaffected fiber fractions with addition of SC.

4.4. In vitro rumen fermentation kinetic

Improved ME, MCP and GY_{24} were observed with the HCP ration. Rations with high protein content provide ruminal microflora with the essential nutrients for its activity. The highly activity reflected on higher GP, higher microbial protein synthesis and higher degradability. This can be generalized for the effect of SC addition on the fermentation activity. Mao et al. (2013) and Elghandour et al. (2014) showed that addition of SC increased ME. They returned their results to the high activities of microbes in the rumen as a result of produced growth factors for microbial growth and activity in the rumen, and to the ability of SC to provide conducive anaerobic conditions to microbial growth (Mosoni et al. 2007).

Result of PF_{24} reflects decreased conversion of degraded substrate into microbial biomass (Harikrishna et al. 2012). Elghandour et al. (2014) showed that addition of SC decreased PF from different poor quality roughages.

5. Conclusions

The high crude protein rations increased gas production and decreased methane production *versus* the low crude protein ration. Addition of *S. cerevisiae* improved ruminal fermentation kinetics with reducing methane production. The commercial *S. cerevisiae* cultures of Biocell F53® and Biosaf SC47® addition at rate of 2 mg/g DM improved fermentation kinetics and nutrients degradability.

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Table 1. Ingredients and chemical composition (g/kg DM) of total mixed rations of different crude protein concentrations

	LCP	HCP
Ingredients		
Ground corn grain	302	228
Ground sorghum grain	280	280
Soybean meal	113	187
Corn stover	250	250
Cane molasses	30	30
Minerals[1]	25	25
Chemical composition		
Organic matter	935	931
Crude protein (N × 6.25)	130	157
Neutral detergent fiber	367	355
Acid detergent fiber	139	140
Hemicellulose	228	215
Metabolizable energy (Mcal/kg)[2]	2.68	2.51

LCP, low crude protein; HCP, high crude protein

[1]Minerals

[2] Calculated according to NRC (2001)

Table 2. *In vitro* rumen gas and methane emission during 48 h of rumen incubation of two mixed rations as affected by different levels of three commercial *Saccharomyces cerevisiae* cultures (mg/g DM)

Ration	Yeast culture	Yeast dose (mg/g DM)	GP parameters			*In vitro* GP (mL/g DM) at:								CH_4 (mL/g DM) at:	
			b (mL/g DM)	*c* (/h)	*L* (h)	2 h	4 h	6 h	8 h	10 h	12 h	24 h	48 h	24 h	48 h
HCP	Control	SC0	359.5	0.031	0.72	20.9	40.6	59.1	76.5	92.9	108.4	183.6	272.6	27.0	57.3
	Biocell® F53	SC2	409.6	0.026	0.40	20.7	40.2	58.8	76.5	93.2	109.0	188.6	289.3	38.8	66.6
		SC4	398.1	0.028	0.92	21.4	41.6	60.7	78.8	95.9	112.0	192.1	290.6	34.2	68.2
	Procreatin 7®	SC2	312.6	0.052	0.55	31.2	59.2	84.3	106.9	127.3	145.5	222.6	285.7	42.0	53.8
		SC4	309.8	0.051	0.49	30.3	57.6	82.3	104.6	124.6	142.7	219.7	283.5	23.6	96.1
	Biosaf® SC47	SC2	428.8	0.024	1.14	19.8	38.8	56.8	74.0	90.4	106.0	185.5	290.2	40.3	54.9
		SC4	401.3	0.026	0.57	20.2	39.4	57.6	74.8	91.2	106.8	184.8	283.9	36.5	64.2
LCP	Control	SC0	309.2	0.032	1.47	19.3	37.3	54.1	69.9	84.7	98.5	164.9	240.7	58.0	141.1
	Biocell® F53	SC2	362.1	0.042	1.16	29.0	55.7	80.2	102.7	123.4	142.4	228.3	311.9	143.2	164.6
		SC4	377.7	0.019	1.52	14.0	27.5	40.4	52.9	64.9	76.5	137.3	224.3	63.8	112.1
	Procreatin 7®	SC2	318.5	0.033	1.02	20.4	39.3	57.0	73.4	88.7	103.0	170.5	245.7	50.6	198.9

	SC4	393.6	0.017	1.35	12.9	25.4	37.4	49.1	60.4	71.2	129.3	215.2	48.5	53.4
Biosaf® SC47	SC2	374.5	0.034	1.35	24.1	46.4	67.2	86.6	104.6	121.4	200.7	288.6	69.8	146.5
	SC4	328.1	0.050	1.39	30.8	58.7	83.9	106.7	127.4	146.0	226.4	295.8	69.9	108.8
Pooled SEM		13.84	0.0035	0.283	2.21	4.11	5.74	7.14	8.33	9.33	12.56	12.44	7.51	9.94
P value														
Ration (R):		0.001	0.656	<0.001	0.071	0.060	0.050	0.041	0.034	0.027	0.006	<0.001	<0.001	<0.001
Yeast culture (P):		<0.001	0.001	0.334	0.788	0.856	0.903	0.921	0.906	0.855	0.213	<0.001	<0.001	<0.001
Yeast dose (D):														
Linear		0.001	0.957	0.750	0.241	0.252	0.263	0.273	0.282	0.291	0.319	0.219	0.411	<0.001
Quadratic		0.023	0.050	0.363	0.005	0.004	0.004	0.004	0.003	0.003	0.002	0.001	<0.001	0.653
R × P		<0.001	<0.001	0.962	<0.001	0.001	0.001	0.001	0.001	0.001	0.006	0.587	0.001	<0.001
R × D		0.020	0.069	0.625	0.126	0.116	0.107	0.100	0.093	0.087	0.062	0.057	0.082	0.118
P × D		0.002	0.001	0.441	0.006	0.006	0.006	0.006	0.006	0.007	0.009	0.025	<0.001	<0.001
R × P × D		0.194	<0.001	0.663	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

b = asymptotic gas production; *c* = rate of gas production; HCP = high crude protein; *L* = the initial delay before gas production begins; LCP = low crude protein.

Means with different superscripts within each column are differ ($P < 0.05$)

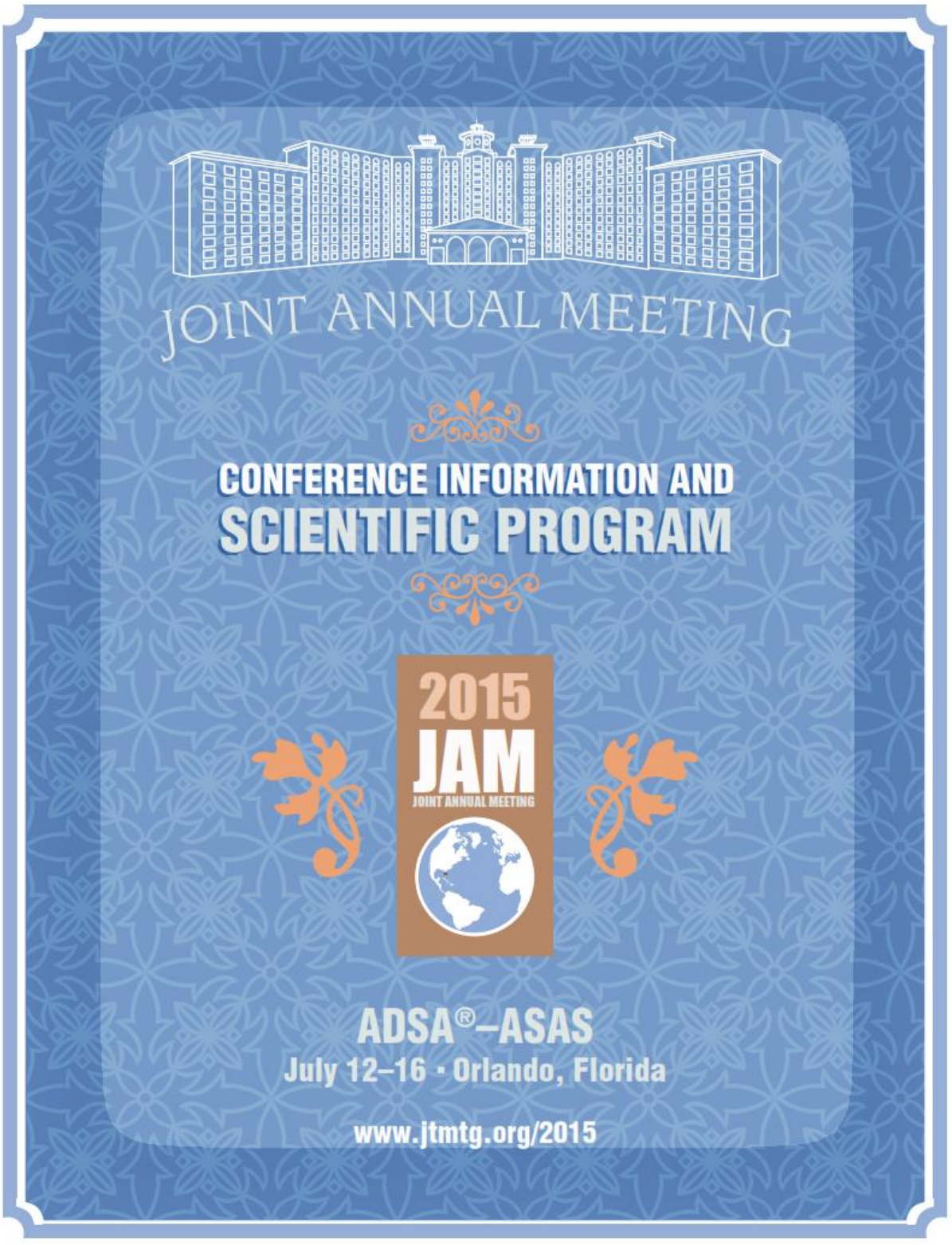
Table 3. *In vitro* fermentation kinetic and degradabilities of two mixed rations as affected by different levels of three commercial *Saccharomyces cerevisiae* cultures (mg/g DM)

Ration	Yeast culture	Yeast dose (mg/g DM)	Fermentation kinetic					Nutrients degradabilities			
			pH	ME	PF_{24}	MCP	GY_{24}	DMD	OMD	NDFD	ADFD
HCP	Control	SC0	6.71	8.09	5.60	619.3	178.7	585.8	550.4	327.5	224.8
	Biocell® F53	SC2	6.77	8.22	5.54	628.6	180.6	317.0	559.2	279.0	201.0
		SC4	6.82	8.32	5.51	635.2	181.4	646.0	565.5	381.7	231.3
	Procreatin 7®	SC2	6.75	9.15	5.33	692.2	187.7	608.3	619.7	323.0	226.7
		SC4	6.74	9.07	5.33	686.8	187.7	721.0	614.5	326.3	224.7
	Biosaf® SC47	SC2	6.85	8.14	5.56	622.9	179.8	633.7	553.8	324.7	225.3
		SC4	6.74	8.12	5.57	621.6	179.6	670.3	552.6	332.7	198.3
LCP	Control	SC0	6.90	7.43	5.76	584.5	173.7	511.6	504.8	346.5	222.5
	Biocell® F53	SC2	6.96	9.15	5.29	702.9	189.0	248.7	617.5	344.3	222.3
		SC4	6.93	6.68	6.08	532.8	164.4	491.3	455.7	339.3	225.3
	Procreatin 7®	SC2	6.86	7.58	5.73	594.9	174.8	582.0	514.7	321.7	221.3
		SC4	6.76	6.46	6.21	517.7	161.1	349.0	441.4	327.3	225.7
	Biosaf® SC47	SC2	6.88	8.40	5.50	651.3	182.3	422.7	568.4	300.7	211.7
		SC4	6.65	9.10	5.29	699.5	189.0	578.0	614.2	332.0	228.3

Pooled SEM	0.473	0.342	0.342	0.105	23.50	53.78	22.34	29.02	10.78
P value									
Ration (R):	0.597	0.001	0.001	0.001	0.006	<0.001	0.001	0.656	0.659
Yeast culture (P):	0.473	0.213	0.213	0.065	0.214	<0.001	0.213	0.997	0.758
Yeast dose (D):									
Linear	0.273	0.319	0.319	0.787	0.319	0.387	0.319	0.864	0.832
Quadratic	0.350	0.002	0.002	0.002	0.002	0.001	0.002	0.123	0.370
R × P	0.462	0.006	0.006	0.015	0.006	0.072	0.006	0.764	0.903
R × D	0.524	0.062	0.062	0.019	0.062	0.095	0.062	0.578	0.681
P × D	0.272	0.009	0.009	0.005	0.009	0.002	0.009	0.640	0.492
R × P × D	0.336	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	0.530	0.227

DMD = *in vitro* dry matter disappearance; GY_{24} = gas yield at 24 h of incubation; HCP, high crude protein LCP, low crude protein; MCP = microbial crude protein production; ME = metabolizable energy; OMD = *in vitro* organic matter digestibility; PF_{24} = partitioning factor at 24 h of incubation.

Means with different superscripts within each column are differ ($P < 0.05$)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T233 Velocity-related changes in stride variables of the intermediate gait of the Irish Draught horse of North America. Molly Nicodemus*[1], Rachel Fletcher[1], and Jeannette Beranger[2], [1]*Mississippi State University, Mississippi State, MS,* [2]*Livestock Conservancy, Pittsboro, NC.*

Although originating as a farming breed having a gait derived from the bloodlines of the extinct ambling Irish Hobby horse, the Irish Draught horse today is bred to excel in the sport-horse industry. With around 500 in North America, the breed is on the Livestock Conservancy's (LC) watch list. Study objectives were to determine the relationship between trotting velocities and stride variables. Ten Irish Draught horses selected by the LC and Irish Draught Horse Society of North America were worked at a slow velocity (SV) and fast velocity (FV) at the trot (SV = 4.5 ± 0.2 m/s; FV = 5.2 ± 0.1 m/s). Frame-by-frame analysis using the Ariel Performance Analysis System was performed documenting hoof contact and lift-off for 10 strides for each horse at each velocity with stride variables given as a % of stride duration. Means (SD) were calculated and student's paired *t*-tests were performed ($P = 0.05$). Both velocities demonstrated a leaping diagonal footfall sequence with diagonal limb pairs at hoof contact and periods of bipedal support (FV = 79 ± 4, SV = 87 ± 3%). While neither velocity demonstrated a 4-beat rhythm at hoof contact, the diagonal limbs disassociated at lift-off (4 ± 1%) at the SV with the hind lifting first creating a period of forelimb unipedal support (9 ± 1%). Along with limb support and coupling of diagonal limbs, fore stance duration (FV = 41 ± 3, SV = 48 ± 1%), stride length (FV = 3.8 ± 0.1, SV = 3.2 ± 0.1 m/s), and length of suspension (FV = 21 ± 4, SV = 4 ± 2%) distinguished between velocities ($P < 0.05$). Stride duration (FV = 0.70 ± 0.01, SV = 0.74 ± 0.02 s) and rate (FV = 1.41 ± 0.02, SV = 1.37 ± 0.01 strides/sec) and hind stance duration (FV = 41 ± 4, SV = 43 ± 2%) remained consistent between velocities. The North American Irish Draught horse did not demonstrate an ambling gait at velocities measured. Nevertheless, pattern and timing of the disassociated limbs and resulting limb support are unique compared with breeds that are today used in the breeding of the Irish Draught horse such as the Thoroughbred and Warmblood, and thus, potentially reflecting gait characteristics of the extinct Irish Hobby horse.

Key Words: stride variable, trot, velocity

T234 Influence of fibrolytic enzymes and yeast addition in horse's diet on digestibility, blood chemistry and fecal coliform. M. M. Y. Elghandour[1], A. E. Kholif[2], A. Z. M. Salem*[1], J. C. Vázquez Chagoyán[1], J. S. Martínez Castañeda[1], L. M. Camacho[3], R. Montes de Oca[1], and T. A. Morsy[2], [1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Estado De México, Mexico,* [2]*Dairy Science Department, National Research Centre, Giza, Egypt,* [3]*Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Guerrero, Altamirano, México.*

Improving fibrous feeds utilization ensures low consumption of high-starch grains and may reduce various pathologies. Fibrolytic enzymes (FE) like cellulase and xylanase, and yeast addition can improve fibrous feeds utilization. Therefore, the current study aimed to study the effect of FE (Exp. 1) and yeast (Exp. 2) on the utilization of diet with 11.2% CP and 51.1% NDF. Thirty-two mares of Quarter Horse (450-500 kg BW) were used in a complete randomize design for 15 d, with 10 d for adaptation and 5 d for samples collection. For Exp. 1, mares (n=16) were distributed onto 4 treatments to be fed on the basal diet without FE (control), or plus cellulase at 10 mL/mare/d (CELL), plus xylanase at 10 mL/mare/d (XYL), or plus mixture cellulase and xylanase at 5 ml of each enzyme/mare/d (CX). For Exp. 2, mares (n=16) were distributed onto 4 treatments to be fed on the basal diet without yeast (control), or fed the control diets plus Procreatin 7 (1.5×10^{10} cfu/g *S. cerevisiae*) at 15 g/mare/d (P7), plus Biocell F53 (2×10^{10} cfu/g *S. cerevisiae*) at 11 g/mare/d (F53), or plus Biosaf SC47 (1.5×10^{10} cfu/g *S. cerevisiae*) at 15 g/mare/d (SC47); yeast products were in powder form. Both of enzyme and yeast doses were mix with the 1 kg of concentrate diet at 0400 h. Mares were fed the concentrates twice daily at 0400 and 1600 h, while the forage of oat straw was offered ad libitum at 0500 and 1700 h. Acid insoluble ash concentrations in feed and fecal samples were used for digestibility determination. No effects for FE and yeast were obtained in blood alanine transaminase, aspartate aminotransferase, urea, creatinine, total protein and glucose. Addition of CELL, XYL and CX increased ($P = 0.001$) nutrient intakes from oat straw versus control. Moreover, CELL, XYL and CX increased ($P < 0.05$) digestibilities of DM, OM, NDF and ADF. Enzymes decreased ($P < 0.05$) concentration of fecal coliform. In the contrary, F53 increased nutrients intake of oat and nutrients digestibility ($P < 0.05$) without difference compared with other treatments. Yeast had no effect on fecal coliform concentration. It could be concluded that addition of FE at 10 mL/mare/d or addition of Biocell F53 at 11 g/mare/d improved feed intake and nutrients digestibility without affecting mare's blood parameters.

Key Words: enzyme, horse, yeast

T235 Changes in salivary IgA and nasopharyngeal leukocyte populations in response to prolonged head elevation. Jill M. Bobel*, Megan R. Di-Lernia, Jeffrey R. Abbott, Maureen T. Long, and Lori K. Warren, *University of Florida, Gainesville, FL.*

Prolonged head elevation is thought to be a major contributor to the increased risk of respiratory disease associated with transportation in horses. Prior investigations have focused on immunological changes in the lower respiratory tract. The aim of this study was to characterize the response to head elevation in the upper respiratory tract. Twelve horses (mean ± SEM, 552 ± 10 kg; 11.5 ± 1.4 y) were tethered for 12 h with their heads elevated at a height of 1.5 m to induce physiological stress. While tied, horses had unlimited access to bermudagrass hay and were offered water every 2 h. Each horse underwent head elevation on 4 occasions, each separated by 30 d. When not tied, horses were maintained on pasture forage. Nasopharyngeal flush (NPF) and saliva samples were obtained before head elevation, immediately after (0 h), and 12, 24, and 72 h post head elevation. Mucus content and leukocyte populations were quantified in NPF and IgA was measured in saliva. Data were compared using mixed model ANOVA with repeated measures. NPF samples contained more mucus at 0 h post ($P < 0.02$) compared with pre-head tie samples. Percentage and number of neutrophils in NPF increased at 0 h post ($P < 0.0001$) and the number remained elevated through 72 h ($P = 0.04$). Lymphocytes, monocytes, and eosinophils in NPF increased in number ($P < 0.05$) but decreased in percent ($P < 0.05$) in response to head elevation. While the proportion of these cells normalized by 72 h, the numbers declined to levels lower than pre-head tie values. Percentage of CD8+ T cells and B cells in NPF were lower at 0 h post ($P < 0.05$) and returned to baseline by 12 h. Percentage of CD4+ and the ratio of CD4+ to CD8+ cells increased at 0 h post ($P < 0.01$) and remained elevated through 72 h ($P < 0.05$). Salivary IgA

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14:10 - 14:30	**Title: Dietary xylanase addition and nutrient digestibility, rumen fermentation and duodenal fibre digestion in sheep** **A Z M Salem**, Autonomous University of the State of Mexico, Mexico
14:30 - 14:50	**Title: Effect of *Sacharomyces cerevisiae* and *Sapindus rarak* supplementation in diet based of Oil Palm Frond (OPF) on nutrient digestibility and body weight gain of goat** **Mardiati Zain**, Andalas University, Indonesia
14:50 - 15:10	**Title: Influence of water deprivation on intake and growth performance of Nguni goats** **C T Mpendulo**, University of KwaZulu-Natal, South Africa
15:10 - 15:30	**Title: Effects of the ß-carotene on the growth performance and skin pigmentation of rainbow trout (Oncorhynchus mykiss, W.1729)** **Guluzar Tuna Kelestemur**, University of Firat, Turkey
15:30 - 15:50	**Title: The effects of probiotic supplementation in weaned pigs diet** **Dragan Sefer**, Belgrade University, Serbia
15:50 - 16:10	**Title: The fiber fraction of oil palm trunk treated by ligninase thermostable produced by thermophilic bacteria isolated from hot spring of west Sumatera, Indonesia** **Yetti Marlida**, Andalas University, Indonesia
	Coffee Break 16:10 - 16:25 @ Foyer
16:25 - 16:45	**Title: The effect of types of mold, fermentation duration and palm kernel cake substrate composition on cellulase and mannanase enzyme activities** **Mirnawati**, Andalas University, Indonesia
16:45 - 17:05	**Title: Improving the quality of catfish burger by adding Zataria multiflora Boiss essential oil** **Ozlem Emir Coban**, University of Firat, Turkey
17:05 - 17:25	**Title: Preparation of nutritious and conservable cattle feed block using agricultural wastes** **WAD Nayananjalie**, Rajarata University of Sri Lanka, Sri Lanka
	Panel Discussion

Day 2 August 12, 2015

Hall-04 Raume/Room-7

Session 02

Track 2: Animal Husbandry and Modern Agriculture
Track 3: Animal Diseases
Track 8: Livestock Nutrition Centers
Track 10: Animal Products

Session Introduction

Session Chair: Isabelle Kalmar, Veos Group, Belgium
Session Co-Chair: Albrecht Glatzle, INTTAS, Paraguay

10:00 - 10:20	**Title: Non-specific antiviral components in plasma can contribute to the safety of SDPP towards PEDV** **Isabelle Kalmar**, Veos Group, Belgium
10:20 - 10:40	**Title: Reconsidering Livestock's role in climate change** **Albrecht Glatzle**, INTTAS, Paraguay
10:40 - 11:00	**Title: The efficacy of certain feed additives for the prevention of *Campylobacter* jejuni infection in broiler chickens** **Wafaa Abd El-Ghany**, Cairo University, Egypt
	Coffee Break 11:00 - 11:15 @ Foyer
11:15 - 11:35	**Title: Crossing beef cattle with *Chihuahuan Criollo* cattle is an efficient alternative for beef production on arid environments of northern México** **Ivan A Garcia Glicia**, University of Chihuahua, Mexico
11:35 - 11:55	**Title: Influence of essential oils and organic acids blend on productive performance, immune status and controlling of Clostridium perferingens infections in broiler chickens** **M A Tony**, Cairo University, Egypt
11:55 - 12:15	**Title: Effect of curcumin with and without protexin on performance parameters, serum lipoproteins, cecum micro flora and gut morphology of broiler chickens** **F Ahmadi**, Islamic Azad University, Iran

12:15 - 12:35	**Title: Comparison of mathematical models described in situ DM Digestion of alfalfa cuts in sheep** **Evans Obura**, Ataturk University, Kenya
12:35 - 12:55	**Title: Alternatives for inhibition of methanogenesis and enhance fermentation of feeds in the rumen** **Gebrehiwot Tadesse**, Mekelle University, Ethiopia
12:55 - 13:15	**Title: Utilization of saline water by Barbarine lambs in the dry areas under climate change** **Wiem Mehdi El-Gharbi**, INRAT, Tunisia

Panel Discussion

Lunch Break 13:15 - 14:00 @ Restaurant

Posters Session (14:00-15:10) @ Foyer

LSN - 01	**Title: Effects of L-Valine supplementation on performance parameters of weaned piglets** **Malte Lohoelter**, CJ Europe GmbH, Germany
LSN - 02	**Title: Effect of Lactation stage on body condition in rabbits** **Eddy Wilfredo Calle**, Polytechnic University of Valencia, Spain
LSN - 03	**Title: The effect of live yeast (*Saccharomyces cerevisiae*) on in-vitro total gas, methane and carbon dioxide production of diet containing 50% oat straw in horses** **A Z M Salem**, Autonomous University of the State of Mexico, Mexico
LSN - 04	**Title: Feed composition at the onset of feeding behaviour influences slaughter weight in rabbits** **Tehya Read**, INRA Genphyse, France
LSN- 05	**Title: Some metal levels of organic milk and dairy products consumed in Turkey** **Emine Baydan**, Ankara University, Turkey
LSN- 06	**Title: Effects fo selection for Residual variance of litter size on body conditionin Rabbit does** **Eddy Wilfredo Calle**, Polytechnic University of Valencia, Spain
LSN- 07	**Title: Effects of high fibre and its source on the growth and slaughter performance of pigs fed maize soybean diets fortified with Roxazyme® G2** **F Fushai**, University of Venda, South Africa

Award Ceremony & Closing Remarks (15:10-15:25)

Coffee Break 15:30 - 15:40 @ Foyer

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The effect of live yeast (*Saccharomyces cerevisiae*) on in-vitro total gas, methane and carbon dioxide production of diet containing 50% oat straw in horses

A Z M Salem[1], Mona M Y Elghandour[1], Juan C Vázquez Chagoyán[1], Jose S Martínez Castañeda[1], Ahmed E Kholif[2], Luis M Camacho[3] and E Nicholas Odongo[3]
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The effect of live yeast (*Saccharomyces cerevisiae*) of three products incubated with equine fecal of horses previously fed the same products on fecal *in vitro* fermentation of a diet contained oat straw and concentrate mixture (1:1) as a substrate was evaluated. Three products of *S. cerevisiae* of Bio-cell F53 (YP53), Procreatin 7 (YP7) and Biosaf SC47 (YP47) were *in vitro* evaluated at 0, 2 and 4 mg/g DM. Fecal inoculums were collected from sixteen horses mares of Cuarto de Milla fed the same concentrate (restricted amount daily) and oat straw (ad libitum) and supplemented with yeast (g/animal/ day) at 0 (control-without yeast), 11 (Biocell F53), 11 (Procreatin 7), and 15 (Biosaf SC47), for 15 days. Gas Production (GP), CH_4 and CO_2 productions were measured at 2, 4, 6, 8, 10, 12, 24 and 48 h post-incubation. Interactions were occurred ($P<0.05$) between fecal type and yeast product for GP, CH_4 production and fermentation parameters. The dose of 2 mg/g DM linearly increased the asymptotic GP ($P=0.021$) and GP during the first 12 h of incubation ($P<0.05$). The product YP53 at 4 mg/g DM decreased ($P=0.028$) CH_4 production by 78% at 24 h. Two mg/g DM of YP53 increased ($P<0.05$) improved fermentation kinetic parameters. It could be concluded that fecal type has an effect on fermentation processes of horse's diets. The product YP53 increased GP, CH_4 and fermentation kinetics at the dose 2 mg/g DM with decreasing CH_4 production by 78% at 4 mg/kg DM at 24 h of incubation.

Biography

A Z M Salem had his PhD in 2002 from Faculty of Veterinary, Leon University, Spain. Now he is working as a Professor Researcher at Faculty of Veterinary Medicine, Autonomous University of the State of Mexico, Mexico. He is specialist in Ruminant Nutrition and working with using the tree leaves extracts, exogenous enzymes, yeasts, As feed additives in animal nutrition. Salem has a lot of research papers published in ELSEVIER, SpringerLink, Wiley, with some text books as well as registered patents in his field of research. He is currently in the editorial board of two international indexed journals in the JCR with impact factor (Journal of Integrative Agriculture and Animal Nutrition and Feed Technology) and he is a reviewer in a lot of scientific international journals.

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Notes:


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Elghandour MMYa, Germán BRb, Kholif AEb, Salem AZMa*, Vázquez Chagoyan JCa, Montes De Oca Jiménez R3, Solorio LJSc, Garcia EAc.
por su valiosa participación como ponente en el
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Dr. Sergio Raúl Fernández Tinoco
Presidente AMENA 2014-2015
Dr. Gerardo Mariscal Landín
Presidente del Comité Científico 2014-2015

VIII.- DISCUSIÓN GENERAL

La presente investigación estudió los efectos del uso de diversas cepas de la levadura *Saccharomyces cerevisiae* como probiótico sobre la degradabilidad tanto de forraje de baja calidad como de dietas integrales con diferente concentración de proteína cruda en la alimentación animal. A las muestras de forraje y/o de dietas balanceadas se les añadió la levadura y se les inoculó, ya sea, líquido ruminal de ganado bovino productor de leche o un preparado a base de heces equinas, para después incubarlas *in-vitro*. A las muestras se les analizó la fermentación a través de la producción de gas en general y metano en particular, la degradabilidad de la materia seca, de la fibra detergente neutro, y de la fibra detergente ácido, así como el tiempo de retraso para iniciar la fermentación (tiempo lag*)*.

La adición de *S. cerevisiae* provocó un aumento en la producción de gas en la mayoría de los tratamientos. En el experimento de Elghandour *et al*. (2014b), la fermentación del rastrojo de maíz usando inóculo fecal de caballos, en presencia de *S. cerevisiae* redujo la tasa de producción de gas y aumentó la asintótica de producción de gas *in vitro* en comparación con las otras especies forrajeras (paja de avena, bagazo de caña de azúcar, pasto llanero, pasto Taiwán, paja de sorgo y pasto estrella). La respuesta del *S. cerevisiae* depende del tipo de alimentación, composición de alimentos, métodos de aplicación, dosis y en general de la interacción dieta-levadura (Patra 2012; Elghandour *et al*. 2014a,b). Elghandour *et al*. (2014a) observó que los parámetros de producción de gas, incluyendo la asintótica de produccion de gas, la tasa de producción de gas y el retardo inicial antes de la fermentación de éste (es decir, tiempo de retraso) variaron entre rastrojo de maíz, paja de avena, bagazo de caña de azúcar y paja de sorgo; con el aumento de la producción y aumento de la asintótica de producción de gas y el tiempo lag con rastrojo de maíz y paja de avena en comparación con bagazo de caña de azúcar y paja de sorgo, confirmando los hallazgos de que los alimentos fibrosos representan una importante fuente de nutrientes paro los equinos en zonas donde escasean los alimentos de buena calidad.

La fermentación del alimento o la dieta produce gases en cantidades diferentes dependiendo de la composición química de los mismos. La producción de gas depende de la disponibilidad de nutrientes para los microorganismos del rumen (Elghandour *et al.,* 2015). La fermentación de los carbohidratos produce una mezcla

de gases en el rumen compuesta principalmente de hidrógeno, dióxido de carbono y metano, con cantidades relativamente pequeñas de gases menores producidos a partir de la fermentabilidad de la proteína en comparación con los carbohidratos (Makkar *et al.,* 1995). La disponibilidad de nutrientes para los microorganismos del rumen estimula la degradabilidad de los diferentes nutrientes (Paya *et al.,* 2007). La levadura de *S. cerevisiae* tiene la capacidad de mejorar la producción de gas disminuyendo la producción de metano, amoníaco y dióxido de carbono reduciendo sus efectos negativos sobre el medio ambiente.

La inclusión de levadura se correlaciona con la disminución del tiempo lag (retraso para el inicio de la fermentación) en la mayoría de los estudios. Elghandour *et al*. (2014b) observó que la adición de *S. cerevisiae* reduce linealmente el tiempo lag para rastrojo de maíz, bagazo y hojas de caña de azúcar, así como para hojas de pastos Taiwán y estrella, en comparación con las hojas de mazorca de maíz, pasto llanero. Newbold *et al.* (1996) explica este fenómeno basado en la actividad respiratoria de la levadura que secuestra O_2, provocando condiciones favorables para las bacterias anaerobias. Además, el *S. cerevisiae* contiene pequeños péptidos y otros nutrientes que favorecen la proliferación de bacterias celulolíticas ruminales predominantes (Callaway y Martin, 1997).

En la mayoría de los casos, el *S. cerevisiae* incrementa la producción de gas de manera dosis dependiente, sin embargo Elghandour *et al*. (2014b) observaron que el efecto de dosis diferentes de *S. cerevisiae* con diferentes especies forrajeras es insignificante para producciones de gas *in vitro*. Por otro lado, Mao *et al.* (2013) y Elghandour *et al.,* (2014a) encontraron efectos positivos de *S. cerevisiae* sobre la fermentación ruminal. Las diferencias encontradas entre los estudios de Elghandour *et al.,* (2014a y b) pueden deberse a los sustratos utilizados o a la cepa de *S. cerevisiae* utilizada. En estos estudios se compararon los forrajes de rastrojo de maíz, paja de avena, bagazo de caña de azúcar y paja de sorgo, que fueron incubadas con inóculo de líquido ruminal, de vacas pardo Suizo, pero en Elghandour *et al*. (2014a), se utilizó *S. cerevisiae* cepa I-1077, (LEVUCELL® SC20; Lalleland, Montreal, QC, Canadá) como probiótico, en tanto que en Elghandour *et al*. (2014b) se utilizó la cepa Procreatin 7 (Safmex/Fermex S.A. de C.V. Toluca, México) de *S. cerevisiae.*

Para comparar el efecto de diferentes productos de levadura, Elghandour *et al*. (2016) compararon tres cultivos comerciales de *S. cerevisiae* (Biocell F53®, Procreatin 7® y Biosaf Sc47®) a diferentes dosis de proteina cruda (PC) (13% vs 16%). Biocell F53® y Biosaf Sc47® incrementaron la asintótica de PG tanto en niveles altos como bajos de PC, encontrándose un mejor efecto con las dosis de 2 mg de levadura/g MS de sustrato alto en PC y la dosis de 4 mg de levadura/g de MS de sustrato de bajo contenido de PC. Los resultados sugieren que la eficiencia de producción de gas puede estar relacionada con la naturaleza de cada cultivo y el contenido de células vivas y otros nutrientes en la preparación del probiótico.

La respuesta a diferentes dosis de levadura varió entre los estudios. Esto puede deberse a la naturaleza de los diferentes procedimientos utilizados *in vitro* en cada experimento y también debido a la naturaleza de la técnica *in vitro*. Lila *y cols.* (2004) observaron respuestas variables con *S. cerevisiae* sobre la fermentación ruminal *in vitro*. La capacidad de la levadura para proporcionar nutrientes importantes y cofactores nutricionales, para estimular las actividades microbianas (Callaway y Martin, 1997), la capacidad para atrapar el exceso de oxígeno (Jouany, 2001) y la capacidad para proporcionar biotina y otras vitaminas (tiamina) (Akin y Borneman, 1990) son las posibles razones de la mejora en la fermentación ruminal y el aumento de la producción de gas. Además, las células *S. cerevisiae* pueden proporcionar un punto focal para el desarrollo de un grupo de población microbiana estable (Jouany , 2001).

En otro estudio realizado por Elghandour *et al*. (2016) para evaluar el efecto de la *S. cerevisiae* sobre la fermentación *in vitro* se demostró que esta puede ser afectada por el contenido de proteína cruda (PC) en la ración (13 vs 16% PC); se encontró que dietas conteniendo 16% de PC tuvieron mayor asintótica de PG. Al aumentar el contenido de proteína se incrementa la producción de gas, aunque la fermentación de proteínas produce relativamente poco gas en comparación con la fermentación de carbohidratos (Makkar *et al*. 1995). Al aumentar el contenido de proteína proporcionalmente se aumenta la disponibilidad de nutrientes para los microorganismos del rumen lo que sugiere que éstos pueden reproducirse con mayor eficiencia (Elghandour *et al*. 2015b). *S. cerevisiae* tiene la capacidad de reducir la producción de amoníaco en el rumen (Hristov *et al*. 2013) debido a la disminución de

la degradación de proteínas y la disminución de la excreción de N global por el animal, lo que contribuye a la disminución de las emisiones de amoníaco a partir de estiércol de ganado (Mao *et al*. 2013). La consecuencia directa de la disminución de la degradación de las proteínas es un incremento de proteinas de paso en el rumen de que pueden ser absorbidas y metabolizadas como proteína verdadera en el abomaso e intestino delgado.

Los experimentos de Salem *et al*. (2015) revelaron que el *S. cerevisiae* tiene la capacidad de disminuir la producción del metano y bióxido de carbono. Sin embargo en los experimentos realizados por Elghandour *et al*. (2014a) se obtuvo un aumento de producción de CH_4. En este estudio se observó una producción de CH_4 insignificante durante las primeras 12 h de incubación, seguido de un aumento rápido hasta alcanzar su pico de concentración al final de la incubación. La producción de gas dio inicio desde temprano en la incubación y estuvo constituido principalmente de H_2, CO_2 y CH_4. Esta situación refleja la naturaleza de los gases producidos durante las diferentes horas de la incubación. Elghandour *et al*. (2016) obtuvieron mayor producción de CH_4 con Procreatin 7® tanto en dietas altas como bajas de PC. Este resultado podría explicarse por un aumento en la proporción de proteínas en la ración, que cambia la concentración de ácidos grasos de cadena corta, de tal manera que se forma menos acético y más propiónico, y por lo tanto, el suministro de hidrógeno para la metanogénesis es limitado (Polyorach *et al*. 2014) por la reducción de la población por protozoos (Iqbal *et al*. 2008).

El método de la aplicación del *S. cerevisiae* puede afectar la cinética de fermentación (Elghandour *et al.,* 2014a) dependiendo del número de células vivas metabólicamente activas (Dawson *et al.,* 1990). La dosificacion directa o la preincubación son los métodos más comunes. La adición directa de levadura puede mejorar la producción de gas *in vitro* así como la cinética y el perfil de fermentación en comparación con el método de pre-incubación dado que se garantiza la viabilidad de las células de la levadura (Elghandour *et al.,* 2014a). Elghandour *et al.,* (2014a) demostraron que la adición directa de *S. cerevisiae* mejoró la tasa de producción de gas reduciendo el retardo inicial antes de que comience la producción de gas en comparación con el método de pre-incubación en el que se adiciona la levadura a las 72 h previas. Hay pocos reportes disponibles acerca del efecto del método de

aplicación de *S. cerevisiae* sobre la cinética de gas *in vitro* y el perfil de fermentación ruminal. Sin embargo, el método de aplicación de *S. cerevisiae* depende del número de células vivas metabólicamente activas que estimulen la fermentación ruminal (Dawson *et al*. 1990). El método de aplicación directa puede asegurar la viabilidad de las células de *S. cerevisiae* lo que resultanta en mejoras en producción de gas y la cinética de fermentación ruminal en comparación con el método de pre-incubación. Elam *et al.* (2003) mostraron que la adición directa implica una modificación favorable de la microflora gastrointestinal.

Elghandour *et al*. (2014a) obtuvo un incremento de la degradabilidad *in vitro* de materia seca (MS), fibra detergente neutro (FDN) y fibra detergente acida (FDA) en el rastrojo de maíz, paja de avena, bagazo de caña de azúcar y paja de sorgo. Además, demostró que con la dosis de 12 mg levadura /g de MS se obtuvieron los valores más altos de digestibilidad de MS, energía metabolizable, DMO y ácidos grasos volatiles. La adición de *S. cerevisiae* aumentó la producción de ácidos grasos de cadena corta y la concentración de energía metabolizable (Mao *et al.,* 2013; Elghandour *et al.,* 2014a). El aumento de producción de ácidos grasos de cadena corta y la concentración de energía metabolizable están asociados con el aumento de las actividades de fermentación ruminal en el rumen. La adición de levaduras están asociadas a la reducción del factor de partición después 24 h (FP_{24}) en la mayoría de los casos. Un menor PF_{24} refleja una menor conversión del sustrato degradado en proteína microbiana (Harikrishna *et al.,* 2012). Elghandour *et al*. (2016) mostró que la ración de 16% de PC tuvo mayores aumentos de EM, PCM, YG_{24}, DMD y DMO con decrementos de FP_{24} en comparación con dietas de 13% PC. Dietas con alto contenido de proteína proporcionan nutrientes esenciales a la microflora ruminal para su actividad. Además, otras razones para el aumento de la flora microbiana son la disponibilidad de factores de crecimiento producidos por otros microorganismos con elevada actividad en el rumen y a la capacidad de *S. cerevisiae* para proporcionar condiciones anaeróbicas para el crecimiento de dichos microorganismos (Mosoni *et al*. 2007).

En la mayoría de los casos, con la adición de levaduras se observó un incremento en la producción de proteína microbiana (Elghandour *et al.,* 2014a,b; Salem et al., 2015).

Como ha sido argumentado, la suplementación con cultivos de levadura en la alimentación de equinos, puede incrementar el número de bacterias que utilizan lactato lo que resulta en un aumento de pH cecal (Lattimer *et al.,* 2007). La mejoría de la digestión de forrajes de baja calidad, con suplementos de levadura en caballos ya ha sido reportada (Morgan *et al.* 2007). El modo de acción de la levadura puede ser aplicado a los caballos ya que las poblaciones microbianas digestivas entre el rumen y el intestino posterior del caballo tiene grandes similitudes (Jouany *et al.,* 2009). La levadura tiene la capacidad de sobrevivir y de transitar a través del tracto digestivo hasta el ciego y colon mejorando la utilización de piensos (Jouany *et al.,* 2008). Además, los cultivos de levadura pueden proporcionar mejores condiciones ambientales microbianas en el intestino delgado y aumentar el número total de microorganismos (Morgan, 2006). La suplementación de *S. cerevisiae* a la dieta de los caballos puede aumentar la población microbiana y mejorar la digestibilidad de nutrientes (Salem *et al.,* 2015; Medina *et al.,* 2002; Lattimer *et al.,* 2005) y mantener en niveles óptimos el pH cecal (Medina *et al.,* 2002; Hall *et al.* 2005).

Los experimentos realizados en esta tesis doctoral sugieren que el uso de levaduras puede mejorar la digestibilidad de la dieta tanto en rumiantes como en equinos y que además reduce la producción de metano. Estos hallazgos apuntan a que se realicen estudios *in vivo* con la suplementación de levaduras a la dieta de rumiantes y equinos para validar los resultados encontrados en el presente trabajo.

IX.- CONCLUSIONES

1- Los resultados de los diversos experimentos realizados sugieren que la adición de *S. cerevisiae* puede mejorar la fermentación ruminal de forrajes de baja calidad
2- El uso de *S. cerevisiae* mejoró la cinética de fermentación ruminal y producción de gas *in vitro* y redujo la producción de metano.
3- Dietas con alto contenido de proteína cruda produjeron mayor cantidad de gas *in vitro* y disminuyeron la producción de metano en comparación con dietas con bajo contenido de proteína cruda.
4- El efecto de *S. cerevisiae* en la fermentación de alimentos depende de la composición química de estos, especialmente los contenidos de fibras y proteína cruda.
5- *S. cerevisiae* constituye un importante aditivo alimenticio que mejora el valor nutritivo del alimentos para rumiantes y equinos en las zonas áridas donde escasean los alimentos de buena calidad

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