UNIVERSIDAD AUTÓNOMA DEL ESTADO DE MÉXICO

MAESTRÍA Y DOCTORADO EN CIENCIAS AGROPECUARIAS Y RECURSOS NATURALES

EFICIENCIA DEL USO ENZIMAS EXÓGENAS Y SUS IMPACTOS SOBRE EL METABOLISMO RUMINAL, LA DIGESTIBILIDAD Y RESPUESTA PRODUCTIVA EN RUMIANTES

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LAURA HAYDÉE VALLEJO HERNÁNDEZ

El Cerrillo Piedras Blancas, Toluca, México; 22 de marzo de 2017.

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COMITÉ TUTORIAL
TUTOR ACADEMICO.
DR. ABDELFATTAH ZEIDAN MOHAMED SALEM

TUTOR ADJUNTO.
DRA. RAÚL CUAUHTÉMOC FAJARDO MUÑOZ
DRA. MARÍA DOLORES MARIEZCURRENA BERASAIN

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Está bien celebrar el éxito, pero es más importante prestar atención a las lecciones del fracaso.

Bill Gates.

RESUMEN

EFICIENCIA DEL USO DE ENZIMAS EXÓGENAS Y SUS IMPACTOS SOBRE EL METABOLISMO RUMINAL, LA DIGESTIBILIDAD Y RESPUESTA PRODUCTIVA EN RUMIANTES

Laura Haydée Vallejo Hernández

Para aumentar la biodisponibilidad de los nutrientes contenidos en ingredientes fibrosos se han desarrollado diversas técnicas, entre las que destacan la adición de enzimas fibrolíticas y levaduras de *Saccharomyces cerevisiae* (SC) en dietas para rumiantes. El objetivo del presente fue evaluar el efecto de la adición de aditivos fibrolíticos en dietas para rumiantes sobre las variables de degradación y fermentación *in vitro* e *in situ*, así como la digestibilidad de nutrientes *in vivo*.

Para la prueba *in vivo* se utilizaron cuatro borregos machos Rambouillet (39 ± 1.8 kg de PV), con cánulas permanentes en rumen y duodeno distribuidos en un diseño cuadrado latino 4×4. Los borregos fueron alimentados con una dieta con 30% rastrojo de maíz sin adición de enzimas (control, XY0), o con la adición de xilanasa en 1 (XY1), 3 (XY3), y 6 (XY6) µl/g MS. Los tratamientos XY1 y XY3 aumentaron el consumo de alimento, mientras que la digestibilidad aumentó con XY6. La digestibilidad de FDN ruminal, el pH ruminal, el nitrógeno amoniacal y el ácido acético aumentaron con las dietas tratadas con xilanasa. La adición de xilanasa a 6 µl/g de MS en una dieta para ovinos Rambouillet mejoró la digestibilidad de la dieta y la fermentación ruminal sin afectar las variables.

En una prueba *in vitro* se evaluó el efecto de diferentes dosis (0, 10, 20, 40 y 80 µg/g MS) de enzima xilanasa (XY) o celulasa (CEL). La producción de gas fue medida a las 2, 4, 6, 8, 10, 12, 24, 36 y 48 h de incubación. Después de las 72 h, la fermentación fue detenida y se registró el pH del medio, el residuo fue filtrado y secado para determinar la degradación materia seca (DIVMS), fibra detergente

neutro (DFDN) y fibra detergente ácido (DFDA). El tipo de enzima afectó (p<0.05) la producción de gas, la DIVMS, degradación de materia orgánica (DIVMO), la concentración de AGV y la proteína cruda microbiana. Las dosis de CEL y XL que tuvo mayor efecto (p<0.05) en la producción de gas en los diferentes horarios fue la de 40 µg/g MS. La adición de CEL o XL disminuyó (p=0.04) el pH del medio. Los resultados sugieren que la dosis de 40 µg/g MS de enzima obtuvo los mejores niveles de producción de gas, lo cual puede mejorar eficazmente la fermentación ruminal.

Por último, en una prueba *in vitro* se determinó el efecto de la adición de XY, SC o XY-SC, sobre las variables de fermentación ruminal en una dieta con 30% rastrojo de maíz. Se evaluaron T1= 0 XY 0 SC, T2= XY (2 µl/g MS), T3= SC (2 mg/g MS) y T4= XY-SC (2 µl/g MS +2 mg/g MS). Se utilizó el inoculo ruminal de 2 bovinos Holstein (600 kg PV), 2 ovinos Rambouillet (65 kg PV) y 2 caprinos criollos (40 kg PV). No se observaron interacciones tipo inóculo × tipo de aditivo (p>0.05) en este estudio. Los aditivos aumentaron (p=0.045) la PG asintótica con inóculo de ovino, sin efecto en los inóculos de caprino y bovino. Los concentraciones de AGV y energía metabolizable aumentaron (p<0.05) cuando los aditivos fueron suplementados a inóculo de caprinos y ovinos. Los aditivos aumentaron (p<0,05) la degradabilidad de MS con inóculo de ovinos y bovinos (p=0.037), y aumentaron (p=0.048) la degradabilidad de la materia orgánica con inóculo de caprinos. La adición de XY, SC o XY-SC no afectaron a la PG total.

ABSTRACT

EFFICIENCY OF THE USE EXOGENOUS ENZYMES AND THEIR IMPACTS ON RUMINAL METABOLISM, DIGESTIBILITY AND PRODUCTIVE PERFORMANCE IN RUMINANTS

Laura Haydée Vallejo Hernández

To increase the bioavailability of the nutrients contained in fibrous ingredients, several techniques have been developed, including the addition of fibrolytic enzymes and *Saccharomyces cerevisiae* yeasts (SC) in diets for ruminants. The objective of the present study was to evaluate the effect of the addition of fibrolytic additives in diets for ruminants on the *in vitro* and *in situ* degradation and fermentation variables, as well as *in vivo* nutrient digestibility.

For the *in vivo* test, four male Rambouillet lambs (39 ± 1.8 kg of PV) with permanent cannulas in rumen and duodenum were used, distributed in a 4 × 4 Latin square design. The lambs were fed a diet containing 30% corn starch without addition of enzymes (control, XY0), or with addition of xylanase in 1 (XY1), 3 (XY3), and 6 (XY6) µl/g MS. Treatments XY1 and XY3 increased feed intake, while digestibility increased with XY6. The digestibility of ruminal NDF, rumen pH, ammoniacal nitrogen and acetic acid increased with diets treated with xylanase. Addition of xylanase to 6 µl/g MS in a Rambouillet sheep diet improved dietary digestibility and ruminal fermentation without affecting blood variables.

In an *in vitro* test the effect of different doses (0, 10, 20, 40 and 80 µg/g MS) of enzyme xylanase (XY) or cellulase (CEL) was evaluated. Gas production was measured at 2, 4, 6, 8, 10, 12, 24, 36 and 48 h of incubation. After 72 h, fermentation was stopped and the pH of the medium was recorded, the residue was filtered and dried to determine dry matter degradation (DIVDM), neutral detergent fiber (DFDN) and acid detergent fiber (DFDA). The type of enzyme affected (p<0.05) gas production, IVDMD, organic matter degradation (IVOMD),

VFA concentration and crude microbial protein. The doses of CEL and XL that had the greatest effect (p<0.05) on gas production at different times were 40 µg/g DM. The addition of CEL or XL decreased (p=0.04) the pH of the medium. The results suggest that the dose of 40 µg/g DM of enzyme obtained the best levels of gas production, which can effectively improve ruminal fermentation.

Finally, in an *in vitro* test the effect of the addition of XY, SC or XY-SC on ruminal fermentation variables in a diet with 30% corn stover was determined. The values T1 = 0 XY 0 SC, T2 = XY (2 µl/g MS), T3 = SC (2 mg/g MS) and T4 = XY-SC (2 µl/g MS + 2mg/g MS) were evaluated. The ruminal inoculum of 2 Holstein steers (600 kg PV), 2 Rambouillet sheep (65 kg PV) and 2 creole goats (40 kg PV) were used. There was no interactions type inoculum × additive type (p>0.05) in this study. The additives increased (p=0.045) asymptotic GP with ovine inoculum, with no effect on goat and bovine inoculum. Concentrations of VFA and metabolizable energy increased (p<0.05) when the additives were supplemented with inoculum of goats and sheep. Additives increased (p<0.05) the degradability of DM with sheep and cattle inoculum (p=0.037), and increased the degradability of organic matter with goat inoculum (p=0.048). The addition of XY, SC or XY-SC did not affect the total GP.

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I. INTRODUCCIÓN

En México las gramíneas, como el rastrojo, la paja de trigo y arroz y el bagazo de caña que representan importantes residuos agrícolas (Vermerris, 2011) que pueden ser utilizados como una fuente de energía abundante y de bajo costo para los rumiantes (Yescas *et al*., 2004), representan el 24% de la materia seca disponible para el consumo animal. La utilización de estos en dietas para rumiantes se ve limitada por el alto contenido lignocelulósico, bajo contenido de proteína cruda (<6%), una mala palatabilidad y baja digestibilidad de los nutrientes (Abdel-Aziz *et al.,* 2015, Togtokhbayar *et al.,* 2015). La digestión de los alimentos fibrosos por los rumiantes es posible, principalmente debido a las enzimas endógenas en el rumen producidas por las bacterias, protozoos y hongos ruminales. Sin embargo, un elevado contenido de fibras impide el acceso de las enzimas ruminales a la pared celular de la planta y reduce la digestibilidad de nutrientes (Kholif *et al.,* 2014).

En investigaciones recientes se sugiere el uso de modificadores del metabolismo ruminal con el fin de mejorar la degradación de las fracciones de fibra y aumentar la biodisponibilidad de algunos compuestos, principalmente carbohidratos simples. Entre estos se encuentran las enzimas fibrolíticas tales como celulasas y xilanasas (Valdes *et al.,* 2015), levaduras vivas (Elghandour *et al.,* 2014) y, en algunos casos, extractos fitogénicos (Bodas *et al.,* 2012; Salem *et al.,* 2014).

El uso de enzimas exógenas en la alimentación de rumiantes mejora la utilización del forraje y reduce la excreción de nutrientes (Mao *et al.,* 2013), incrementa el valor de la alimentación y el rendimiento animal mejorando la degradación de las fibras, aumentando la ingesta y la digestión del alimento (Salem *et al.,* 2015). En el rumen se incrementa la degradación de la proteína de la dieta, que a su vez incrementa la síntesis de proteína microbiana (Yang *et al.,* 1999), se estimula el crecimiento de otros microorganismos proporcionando metabolitos esenciales

como propionato, aminoácidos y vitaminas (Jespersen, 2003) y se mejora la degradación del alimento (Pinos-Rodríguez *et al.,* 2002).

En cuanto a producción animal, se ha demostrado que la adición de enzimas fibrolíticas mejora el estatus energético; en vacas, reducen las concentraciones plasmáticas de β-hidroxibutirato, indicando que la movilización de grasa del tejido adiposo se reduce en lactancia temprana y media (Mendoza *et al.,* 2014). Además de un incremento en la síntesis de proteína microbiana. Se ha reportado incremento en el consumo y digestibilidad de forraje (Pinos-Rodríguez *et al.,* 2002) e incluso aumento en la producción de leche de ovejas alimentadas con la adición de xilanasa (Carro *et al*., 2006). En vacas lecheras, el incremento en la tasa de digestión en la fibra detergente neutra (FDN) equivalente al 10%, permite aumentos de 30% en la energía neta para lactación y de 20% en la producción de leche (Yang *et al*., 2010), en ovinos aumentó la ganancia diaria de peso, el consumo de materia seca (Salem *et al*., 2011) la digestibilidad y la conversión alimenticia (Tirado-Estrada *et al*., 2011).

Sin embargo, la adición de enzimas fibrolíticas no tiene un efecto claro o consistente en cuanto a respuesta productiva (Pinos-Rodríguez *et al*., 2008), algunos estudios han concluido que la adición de enzimas fibrolíticas no sugieren ningún efecto sobre el consumo de alimento ni la digestibilidad de nutrientes en rumiantes (Elwakeel *et al.,* 2007, Dean *et al*., 2013), aún se desconoce el porqué de la variabilidad en la respuesta, no obstante, se han formulado algunas hipótesis que contemplan la actividad enzimática, la forma de aplicación de la enzima, la interacción enzima-sustrato, la estabilidad de la enzima (Dean *et al.*, 2013).

Por otro lado, la adición en dietas para rumiantes de levaduras vivas provenientes, principalmente, de *Saccharomyces cerevisae* han demostrado efectos benéficos en las variables de fermentación ruminal e incrementar la digestión del forraje, esto debido a que las levaduras son una fuente natural de enzimas digestivas, ácidos grasos, vitaminas del complejo B, factores de crecimiento y aminoácidos

(Vázquez *et al.,* 2013). Además, cuando son empleadas en dietas con alto contenido de almidones, incrementan la producción de ácidos grasos volátiles y estabiliza el pH al reducir la producción de ácido láctico (Moloney y Drennan, 1994). Se ha propuesto que la adición de levaduras y enzimas aumenta la digestión del alimento, pero los resultados no son claros en cuanto a la eficacia de estos en dietas altas en grano (Baumann *et al.,* 2004).

Por lo anterior, el presente estudio está enfocado a determinar el efecto de la adición de enzimas exógenas a diferentes dosis y su asociación con una levadura, sobre las variables de fermentación ruminal y la digestibilidad del rastrojo de maíz.

II. REVISIÓN DE LITERATURA

2.1 Pared celular vegetal

Las plantas comprenden paredes celulares primarias y secundarias fortificadas por microfibrillas de celulosa y compuestas por polisacáridos, proteínas, lignina, grupos acetilos, componentes fenólicos. Entre los polisacáridos se encuentran hexosas (glucosa, galactosa y manosa), pentosas (arabinosa y xilosa), 6-deoxi hexosas (ramanosa y fucosa) y ácidos urónicos (galacturónico, glucorónico y 4-O-metil glucóronico (Aman, 1993).

Las paredes celulares primarias contienen celulosa, hemicelulosa (xiloglucanos), pectina (en gramíneas es sustituida por glucoronoarabinoxilano) y proteínas (Vogel, 2008), estos componentes son hidrolizados por enzimas catabólicas especificas (Cuadro 1) (Madigan *et al.*, 2004). Las paredes celulares secundarias están compuestas por celulosa (35-50 %), hemicelulosa (20-35 %) y lignina (10-15 %) (Mendoza *et al*., 2014) que constituyen la mayoría de la masa de la pared celular; la variación depende siempre de la especie vegetal y del estado de desarrollo del forraje (Wyman *et al.,* 2005).

Cuadro 1. Componentes de la pared celular y enzimas que los hidrolizan

Componente	Composición	Enzima catabólica
Celulosa	Polímero de glucosa (β 1,4)	Celulasa (β 1,4 glucanasas)
Almidón	Polímero de glucosa (α 1,4)	Amilasa
Pectina	Polímero de ácido galacturónico	Pectinasa (poligalacturonasa)
Xilano	Heteropolímero de xilosa y otros azúcares (β 1,4 y grupos laterales α 1,2 o α 1,3)	Xilanasa
Sacarosa	Disacárido glucosa-fructuosa	Invertasa

Adaptado de Madigan *et al,* (2004)

La celulosa es el biopolímero más abundante en la tierra (Vermerris, 2011), está formado por unidades repetitivas de glucosa unidas entre los carbonos 1 y 4 en orientación β (Madigan, 2003). La hemicelulosa es el segundo polímero más abundante en la biomasa vegetal, consta de un conjunto de biopolímeros que se caracterizan por una estructura química heterogénea formado de pentosas (xilosa, arabinosa), hexosas (manosa, glucosa, galactosa) y azucares ácidos (Saha, 2003). La lignina es el polímero natural más complejo en relación a su estructura y heterogeneidad, es un polímero ramificado formado por la unión de cuatro alcoholes (Coniferilo, hidroxiconiferilo, cumarílico y sinapílico) (Moore y Jung, 2001).

La hemicelulosa se encuentra asociada a la celulosa y la lignina que contribuyen a la rigidez y flexibilidad de la pared celular vegetal (Cosgrove, 1977). La proporción de estos componentes puede ser el principal factor limitante del consumo, la digestibilidad y la biodisponibilidad de nutrientes para rumiantes; los rastrojos de maíz y pajas de gramíneas contienen paredes celulares secundarias que son rígidas y resistentes a la adhesión y degradación microbiana (Aman, 1993). Li, *et al.* (2015) reporta una correlación negativa entre la proporción y composición de lignina con la degradabilidad biológica de la pared celular ya sea por la microbiota ruminal *in vitro* (Jung *et al.,* 2000) *in vivo* (Jung *et al.,* 1997), así como la hidrólisis por enzimas fibrolíticas exógenas (Oakley *et al.,* 1999).

2.2 Actividad enzimática por microorganismos ruminales

Los rumiantes son una de las especies domésticas mejor adaptadas para una mayor utilización de las paredes celulares vegetales, esto debido a la simbiosis que existe entre el animal y los microorganismos ruminales (Hungate, 1984) los cuales colonizan y digieren las partículas del alimento; la hidrólisis de los componentes de la pared celular mediante proceso de fermentación tiene como producto final a los ácidos grasos volátiles (AGV), que representan la principal fuente de energía para el rumiante (Krause *et al.,* 2003).

Se ha identificado que las bacterias que tienen un papel activo en la degradación de fibra en el rumen son: *Ruminococcus albus, Ruminococcus flavefaciens, Fibrobacter succinogenes* y *Butyrivibrio fibrosolvens*, bacterias altamente xilanolíticas; *Prevotella*, que produce altas cantidades de xilanasa (Stewart *et al.,* 1997). Además, se ha demostrado actividad fibrolítica en hongos, principalmente en *Neocallimastix* sp., (Orpin *et al.,* 1997) y protozoarios (Williams *et al.,* 1997), aunque no se han comprendido bien las rutas metabólicas que utilizan éstos (Devillard *et al.,* 2003).

La actividad enzimática dada por los microorganismos ruminales depende de las condiciones del rumen, principalmente el pH*. Fibrobacter succinogenes* no tolera pH por debajo de 5.9, mientras que *Butyrivibrio fibrosolvens* tolera pH de 5.7 (Montañez *et al.,* 2013). Así, la degradación de fibra en rumen depende del tipo de forraje ofrecido, y de los componentes de su pared celular, pues la rápida fermentación de estos puede dar como resultado un ambiente ácido que afecte a los microorganismos ruminales.

Por lo anterior, en rumen, la digestibilidad total de la pared celular vegetal sólo llega a 65% (Van Soest, 1982), la supuesta incapacidad de los microorganismos ruminales para expresar la combinación apropiada de enzimas para maximizar la digestión del alimento (Krause *et al.,* 2003), es una razón para la investigación de alternativas tales como el uso de enzimas fibrolíticas exógenas.

2.3 Enzimas fibrolíticas exógenas

Las enzimas fibrolíticas exógenas (EFE) son aditivos modificadores del metabolismo ruminal que pueden mejorar la digestibilidad de los componentes fibrosos de la dieta, y de esta manera incrementar la energía digestible para rumiantes (Beauchemin y Holtshausen, 2010). Las EFE generalmente son obtenidas de organismos celulolíticos (Cuadro 2), los más comunes son los cultivos de hongos (*Aspergillus oryzae* y *Saccharomyces cerevisiae*) y de algunos

tipos de bacterias que tienen la capacidad de producir cantidad suficiente de enzimas celulasas y hemicelulasas (Gado y Salem, 2013a), capaces de degradar la pared celular vegetal.

Cuadro 2. Organismos celulolíticos utilizados en dietas para rumiantes

Actividad enzimática	Organismo celulolítico
Celulasa	*Aspergillus niger*
	Trichoderma longibrachiatum (T. reesei, T. viride)
	Humicola insolens
β-glucanasa	*Aspergillus niger*
	Aspergillus aculeatus
	Bacillus lentus
	Bacillus subtilis
	Humicola insolens
	Penicillum funiculosum
	Trichoderma longibrachiatum (T. reesei, T. viride)
Hemicelulasa	*Aspergillus niger*
	Trichoderma longibrachiatum (T. reesei, T. viride)
	Bacillus lentus
	Bacillus subtillis
	Humicola insolens
	Aspergillus aculeatus
Xilanasa	*Aspergillus niger*
	Bacillus lentus
	Bacillus subtilis
	Trichoderma longibrachiatum (T. reesei, T. viride)
	Penicillum funiculosum
	Humicola insolens

Adaptado de Beauchemin *et al,* (2003)

La respuesta enzimática dependerá de la actividad, cantidad o proporción, especificidad de la enzima, la estructura de la pared celular y del método de administración de la enzima (McCallister *et al.,* 2001).

2.3.1 Mecanismos de acción

Montañez *et al.* (2013) señalan que conocer el exacto mecanismo de acción de las EFE es complicado debido a tres factores principales:

- Los forrajes utilizados tienen estructuras complejas.
- Los productos enzimáticos contienen varios tipos de enzimas (mezclas) que tienen diferentes especificaciones y condiciones óptimas para su actividad enzimática.
- El líquido ruminal es un ecosistema con cientos de microorganismos, que tienen actividad enzimática propia.

Sin embargo, Beauchemin *et al,* (2004) proponen tres momentos en los que la respuesta favorable de las enzimas puede ser explicada:

- Previo a la ingesta: las enzimas aplicadas al alimento comienzan la hidrólisis de las paredes vegetales para facilitar la adhesión bacteriana; además se puede facilitar la unión enzima sustrato.
- En rumen: la hidrólisis del sustrato tiene como resultado metabolitos secundarios que pueden ser aprovechados por las bacterias ruminales para su crecimiento (Morgavi *et al.*, 2000) y actividad fibrolítica, actuando en sinergia (Salem *et al*., 2012) para aumentar la hidrólisis de los componentes de la pared celular vegetal (Rode *et al.,* 1999).
- En intestino delgado: las enzimas pueden permanecer viables en el intestino y tener un efecto hidrolítico, sucede cuando hay una alta tasa de pasaje en rumen y las enzimas adheridas al bolo llegan a intestino (Beauchemin *et al.,* 2004).

La mayoría de las enzimas implicadas en la degradación de la celulosa y hemicelulosa son glicosil hidrolasas (Krause *et al.,* 2003). Para la hidrólisis de la

celulosa se requiere de la sinergia enzimática de endoglucanasas, exoglucanasas y β-glucosidasas (Forsberg *et al.,* 1997). La hemicelulosa es hidrolizada por las xilanasas, que actúan sobre el xilano a través de las endo-β-1,4-xilanasas y β-xilosidasas producidas por las endoxilanasas; las estereasas eliminan los grupos acetilo de la estructura principal del xilano (Poutanen *et al*., 1991) (Cuadro 3).

2.4 Uso de enzimas fibrolíticas exógenas en la alimentación de rumiantes

Inicialmente, el uso de EFE era aplicado únicamente en dietas para no rumiantes, se argumentaba que las enzimas podrían ser degradadas por las proteasas secretadas por las bacterias del rumen (Mendoza *et al*., 2014); aunado a esto el pH en el que las enzimas tienen mayor actividad va de 3 a 7 para β-glucanasa y de 6 a 7 para xilanasa (Montañez *et al.,* 2013), mientras que las condiciones normales del rumen de un animal alimentado con ingredientes fibrosos son de un pH cercano a neutro: 6.8 – 7 (Church, 1993).

Sin embargo, la digestibilidad ruminal de la FDN raramente supera el 50%, sobre todo cuando las condiciones del rumen no son favorables para una adecuada actividad fibrolítica, como lo es cuando el rumiante es alimentado con dietas con alto contenido de granos. Por lo cual, en muchos estudios se implementó la adición de EFE en dietas para rumiantes, principalmente en ganado lechero alimentado con dietas con forrajes de alta calidad (alfalfa y ensilado de maíz), observando resultados positivos (Pinos-Rodríguez *et al*., 2003), como una alternativa potencial para reducir los costos de alimentación, al reducir el nivel de inclusión de grano en la ración y afectando las variables productivas (Vargas *et al*., 2013). Sin embargo, resultados inconsistentes y el costo de las EFE han impedido que se establezca una relación clara entre la dosis y método de aplicación de las enzimas y el efecto en las variables productivas (Mendoza *et al*., 2014).

Cuadro 3. Enzimas que hidrolizan la pared celular vegetal

Polímero	Enzima	Sustrato	Enlace que hidroliza	Producto final
Celulosa	Endo-β-1,4-glucanasa	Celulosa	Glucosa-β-1,4	Celu-oligómeros
	Exo-β-1,4-glucanasa	Celulosa en el extremo reducido	Glucosa-β-1,4	Celobiosa
Hemicelulosa	β-glucosidasa	Celobiosa	Glucosa-β-1,4	Glucosa
	Endo-β-1,4-xilanasa	Xilano	Glucosa-β-1,4	Xilo-oligómeros
	β-1,4-xilosidasa	Xilobiosa	Glucosa-β-1,4	Xilosa
	α-L-arabinofuranosidasa	Arabinoxilano	Glucosa-β-1,4	Arabinoxilano y arabinosa
	α-glucuronidasa	Glucoronoxilano	α-1-3 o α-1-2	Ácido glucúronico y xilano
	Acetil-xilano esterasa	Acetil xilano ácido ferúlico	Acetil-éster	Acetato y Xilano
	Ácido ferúlico esterasa	Enlaces	Feruloil-éster	Ácido ferúlico y xilano

Adaptado de Beauchemin *et al,* (2003)

Cuadro 4. Efecto de la adición de EFE sobre las variables de fermentación ruminal.

Especie	Sustrato	Producto enzimático	Dosis	Impacto (Con referencia altratamiento control)	Referencia
In vitro					
Caprino	Esquilmos (Arroz, Maíz y Trigo) Forrajes (Alfalfa y pasto Guimu)	Celullase®	12 UI g^{-1}MS	↓ pH del medio 6.2% ↓ Concentración mg L^{-1} de N-NH_3 37.7% ↓ Concentración mmol g^{-1}MS de CH_4 6.26%	Tang *et al.,* 2013
Ovino	85 concentrado 15 heno de alfalfa	ZADO®	5, 10 y 20 mg	Dosis crecientes de enzima ↑ la producción de gas las primeras 12 h de incubación. ↓ Fase Lag (*L*).	López *et al*., 2013
Ovino	Paja de arroz	Xylanase®	15 UI g^{-1} MS	↑ Concentración mmol g^{-1}MS de CH_4	Mao *et al.,* 2013
Bovino	Ensilado de maíz	Sill All®	10 g ton^{-1} (en el ensilado)	↑ Producción total de gas (mL gas g^{-1} MS incubado)	Ruiz *et al.,* 2013
Ovino	Forraje (pasto)	Cellulase Xylanase	7.5UI/500g MS	↑ Producción total de gas (mL gas g^{-1} MS incubado) ↓ Concentración mmol g^{-1}MS de CH_4	Soltan *et al.,* 2013
Caprino	Rastrojo de maíz	Xilanasa	80 mg kg^{-1} MS	↑ Concentración molar de AGV totales 27.5% ↑ Concentración mmol g^{-1}MS de CH_4 11.2%	He *et al.,* 2015

Cuadro 5. Efecto de la adición de EFE sobre las variables de fermentación ruminal. *(Continuación)*

Especie	Sustrato	Producto enzimático	Dosis	Impacto (Con referencia altratamiento control)	Referencia
Ovino	Paja de arroz	Rovabio Excel®	1µL	↑ Tasa de fermentación 21% - 48%	Díaz *et al*., 2013
	Rastrojo de maíz	Xilanasa		↑ La concentración total de AGV 40% - 90%	
	Pasto henificado	Celulasa		↓ La proporción acetato:propionato	
In situ					
Ovino	35% rastrojo de maíz	Fibrozyme®	1 g kg^{-1} de forraje	↓ 24.34% la concentración molar de AGV totales a las 12 h postprandial	Yescas *et al.,* 2013
Bovino	Forraje 40%	Fibrozyme®	15 g $animal^{-1}$ $día^{-1}$	↑ Flujo de Nitrógeno en duodeno 10%	López *et al.,* 2006
				↑ Cinética ruminal (kd) de FDN 35.29%	
Ovino	Forraje 35%	Fibrozyme®	1 g kg^{-1} de forraje	↑ 24.34% la concentración molar de AGV totales a las 12 h	Yescas *et al.,* 2004
Bovino	Forraje 45%	Promote®	4 g $animal^{-1}$ $día^{-1}$	↓ 12.58% la concentración mg dL^{-1} de N-NH_3	Dean *et al.,* 2013
				↓ 20.14% la concentración molar de	

Cuadro 6. Efecto de la adición de EFE sobre las variables de producción animal

Especie	Sustrato	Producto enzimático	Dosis	Impacto (Con referencia altratamiento control)	Referencia
In vitro					
Caprino	Esquilmos (Arroz, Maíz y Trigo) F	Celullase®	12 UI g^{-1}MS	↑ % DIVMS hasta 76% ↑ % DIV FDN hasta 37%	Tang *et al.,* 2013
Ovino	15% heno de alfalfa	ZADO®	5, 10 y 20 mg	↑ % DIVMS de 14 a 16%	López *et al*., 2013
Bovino	Forraje 60% (Rastrojo de maíz y heno de alfalfa)	Extracto de *Cellulomonas flavigena*	0, 2.5, 7.5 y 12.5 mL kg^{-1} MS	Dosis crecientes de enzima ↑ % DIVMS a las 6 h de incubación e ↑ % DIVFDA a las 12, 24 y 72 h.	Torres *et al*., 2013
Bovino	Heno de pasto Guinea	Promote® Biocellulase X-20® Biocellulase A-20®	0.5x, 1x y 2x la dosis recomendada por el fabricante	En dosis crecientes con todos los productos enzimáticos ↓ % digestibilidad *in vitro* de MS (7%), FDN (9.17%) y FDA (4.54%).	Dean *et al*., 2013
Ovino	Paja de arroz, DDGS y combinación de ambas (ensilados)	ZAD®	3 mL kg^{-1} MS (en el proceso de ensilado)	↑ % DIVMS 51.58% (Paja de arroz), 13.37% (DDGS) y 78.51% (Combinación). ↑ % DIV FDN 17.11% (Paja de arroz), 13.43% (DDGS) y 26.12% (Combinación).↑ % DIV FDA 17.01% (Paja de arroz), 11.42% (DDGS) y 9.56% (Combinación)	Gado *et al.,* 2013

Cuadro 7. Efecto de la adición de EFE sobre las variables de producción animal *(Continuación)*

Especie	Sustrato	Producto enzimático	Dosis	Impacto (Con referencia altratamiento control)	Referencia
Ovino	Paja de arroz	Cellulase	7.5 UI g^{-1} MS	↑ % DIVMS y FDA	Mao *et al.,* 2013
		Xylanase	15 UI g^{-1} MS	↑ Concentración mmol g^{-1}MS de CH_4	
Bovino	Forraje 50%	Fibrozyme®	2 g kg^{-1} MS	↑ % DIVMS 2.5%	Moreno *et al.,* 2007
				↑ % DIV FDA >10%	
Caprino	Rastrojo de maíz	Xilanasa	80 mg kg^{-1} MS	↑ % DIVMS 11.7%	He *et al.,* 2015
				↑ % DIV FDN 36.2%	
Bovino	Heno de alfalfa	Fibrozyme®	500 mg kg^{-1} MS	↑ % DIVMS 45.6%	Pinos *et al.,* 2002
				↑ % DIV FDN 106%	
In situ					
Ovino	60% paja de avena	Fibrozyme®	5 g kg^{-1} de forraje	↑ Digestibilidad *in situ* a las 48 h de incubación	Lara *et al.,* 2013
Bovino	Forraje 40%	Fibrozyme®	15 g $animal^{-1}$ $día^{-1}$	↑ Digestión ruminal de FDN 27.8%	López *et al.,* 2006
				↑ Digestión ruminal de FDA 28.6%	
				↑ Digestión ruminal de MO 4.8%	
Ovino	Forraje 35%	Fibrozyme®	1 g kg^{-1} de forraje	↑ Digestibilidad *in situ* de FDN a las 12 h de incubación	Yescas *et al.,* 2004

2.5 Uso de levaduras en la alimentación de rumiantes

Las levaduras son uno de los probióticos más utilizados en la alimentación de rumiantes, se han observado efectos benéficos, los cuales son atribuibles al aumento en la hidrólisis de la celulosa en rumen, del aumento en el crecimiento bacteriano y por tanto incremento en el flujo de proteína microbiana hacia el intestino (Newbold, 2006).

En cuanto al ecosistema ruminal, se ha demostrado que la adición de levaduras tiene un efecto de regulación del pH del rumen (Moloney y Drennan, 1994), captando metabolitos intermedios, de esta manera también afecta la metanogénesis, al disminuirla (Reynoso *et al.,* 2010), además de ser una fuente de vitaminas y ácidos orgánicos (ácido málico) (Vázquez *et al.,* 2013), lo anterior favorece a la actividad óptima de la microbiota ruminal, incrementado la energía disponible para el rumiante (Fonty y Chaucheyras, 2006) ya que la concentración de AGV totales aumenta (Lila *et al*., 2004, García *et al*., 2000). Sin embargo, la respuesta ruminal depende de la dosis de levadura, tipo de cepa utilizada y la interacción de estas con la dieta (Sales, 2011).

III. JUSTIFICACIÓN

Actualmente, el uso de granos de cereales y semillas de oleaginosas en dietas para rumiantes se ve limitada por factores como disponibilidad, precio y competencia de los ingredientes para la alimentación de no rumiantes. Ante esto, surge la necesidad del uso de subproductos y esquilmos agrícolas, los cuales, a pesar de ser económicamente viables, tienen como desventaja mala palatabilidad y un perfil nutricional bajo debido a que los nutrientes están ligados a las estructuras de la pared celular vegetal, lo que limita su biodisponibilidad y digestibilidad. En la alimentación de rumiantes con dietas que incluyan forrajes de baja calidad, la adición de enzimas fibrolíticas y levaduras puede significar una alternativa para el mejor aprovechamiento de estos productos. Las enzimas fibrolíticas actuarían hidrolizando los enlaces de la pared celular vegetal, aumentando así la biodisponibilidad y la digestibilidad de los nutrientes contenidos, mientras que las levaduras, coadyuvarían en este proceso con su propio sistema enzimático. Un incremento en la disponibilidad de los nutrientes permite, en un primer momento, una mayor actividad de los microorganismos ruminales, traducido como incremento en las variables de la fermentación ruminal, específicamente, la concentración de ácidos grasos volátiles, nitrógeno amoniacal y proteína microbiana; esto, a su vez repercute directamente con las variables de producción animal, entendiendo que a mayor absorción de ácidos grasos volátiles y nitrógeno amoniacal, aumenta la disponibilidad de energía y compuestos nitrogenados para la síntesis de músculo o leche. Por otro lado, una mayor digestibilidad de los nutrientes, disminuye el contenido de estos en heces y la deposición de compuestos contaminantes en el suelo, mitigando así su potencial contaminante.

IV. HIPÓTESIS

La adición de enzimas fibrolíticas en dietas para rumiantes incrementa la hidrólisis de las paredes vegetales, incrementando así los valores de las variables de degradación, fermentación y microbiológicas ruminales, y aumenta la digestibilidad de nutrientes.

V. OBJETIVOS

5.1 Objetivo general

Evaluar el efecto de la adición de enzimas fibrolíticas en dietas para rumiantes sobre las variables de degradación y fermentación *in vitro* e *in situ*, así como la digestibilidad de nutrientes *in vivo.*

5.2 Objetivos específicos

- Determinar el efecto de la adición de xilanasa a una dieta con 30% de rastrojo de maíz sobre las variables de fermentación (concentración de AGV y N-NH_3) y degradación de materia seca *in situ* en borregos Rambouillet.
- Determinar el efecto de la adición de enzimas celulasa o xilanasa sobre la producción de gas, las variables de fermentación (concentración de AGV y N-NH_3) y degradación *in vitro* de rastrojo de maíz.
- Determinar el efecto de la adición de xilanasa y su asociación con levadura de *Saccharomyces cerevisiae* sobre la producción de gas, las variables de fermentación (concentración de AGV y N-NH_3), degradación y microbiológicas ruminales *in vitro,* utilizando inoculo ruminal de bovino, ovino y caprino.

VI. MATERIAL Y MÉTODOS

Todos los procedimientos involucrados en el manejo de los animales con cánula en rumen y duodeno fueron realizados bajo las condiciones estipuladas en la Norma Oficial Mexicana de especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio (NOM-062-ZOO-1999).

Para el desarrollo de este estudio, se llevaron a cabo 3 pruebas, de las cuales se realizó un artículo científico por cada una de ellas, y cuya metodología se describe a continuación:

6.1 Efecto de la suplementación con xilanasa sobre el consumo de alimento, digestibilidad y fermentación ruminal de borregos Rambouillet.

Localización

Los análisis químicos fueron realizados en el laboratorio de Nutrición Animal y la prueba con ovinos, en la Unidad Metabólica del Programa de Ganadería en el Colegio de Postgraduados, carretera Los Reyes-Texcoco, km 36.5, Montecillo, Estado de México. La altitud es 2240 m; el clima (García, 1981) es (Cwo) (w) b (i') (g); clima templado subhúmedo con lluvias en verano, época seca en invierno, una temperatura promedio anual de 15.2 ºC y 650 mm de precipitación promedio anual. El experimento se llevó a cabo durante la época de otoño de 2014.

Animales

Se utilizaron 4 borregos machos raza Rambouillet, con peso de 39.2 ± 1.8 kg, con cánula en rumen (2.5 cm de diámetro interno) y en duodeno (0.8 cm de diámetro interno). Los animales fueron alojados en jaulas elevadas individuales equipadas con bebedero automático con válvula de acero. Se ofreció el alimento experimental (Cuadro 8) *ad libitum*, para ello se consideró como libre acceso si el rechazo representaba el 10% de lo ofrecido. Al inicio del experimento se aplicó a los borregos Ivermectina (Ivomec®-F 1mL 50 kg^{-1} de PV subcutánea), Bacterina

(Covexin® 10 1mL animal^{-1} intramuscular) y vitaminas A, D y E (Vigantol® ADE, 1 mL animal^{-1} intramuscular).

Dieta

La dieta tenía como base el 30% de rastrojo de maíz, los componentes se muestran a continuación (Cuadro 8).

Cuadro 8. Composición (%) de las dietas experimentales en base seca.

Ingredientes	%
Rastrojo de maíz	30
Grano de sorgo molido	52
Pasta de soya	6
Melaza	8
Urea	4

Diseño experimental

Los animales fueron distribuidos en un modelo de Cuadrado Latino 4 x 4, donde se evaluaron dosis de 0, 1, 3, 6 µl de xilanasa /g MS en una dieta experimental

Periodos experimentales

Los periodos experimentales fueron de 21 días. Del día 1 a 15 se llevó a cabo la adaptación a la dieta experimental y del día 16 al 21 colección de muestras.

Toma de muestras y variables evaluadas

Consumo de materia seca, a partir del día 16 de cada periodo se registró el peso del alimento ofrecido y rechazado, determinando por diferencia de estos el consumo de alimento.

Digestibilidad aparente *in vivo*: Se recolectó y pesó el total de heces durante 24 horas, de los días 16 al 21 de cada periodo. El peso total de las heces fue registrado, una muestra del 10% del total se secó a 55 ºC durante 48 h para

determinar el contenido de materia seca. La digestibilidad aparente se calculó de acuerdo a la ecuación de Merchen (1988).

Nutrientes en líquido de duodeno: Los días 16 y 17 se tomaron 500 g de fluido duodenal a las 4 h postprandial. La muestra se obtuvo destapando la cánula duodenal y dirigiéndola a un frasco ámbar de 100 mL, la muestra obtenida fue conservada en congelación hasta su análisis.

Posteriormente la muestra fue secada a 55 ºC durante 48 h, una vez seca se homogeneizo y se determinó % de proteína, fibra detergente neutro (FDN) y fibra detergente ácida (FDA)

Fermentación ruminal: el día 18 del periodo, se colectaron muestras de rumen, a las 0 h antes de la alimentación, y 3, 6 y 9 h postprandial, para determinar pH, AGV, NH3-N.

Las muestras recolectadas directamente de rumen con sonda, fueron filtradas usando una capa triple de manta cielo, y el pH se midió con un potenciómetro portátil (ORION, modelo SA 210). Posteriormente se colocaron 4 mL de líquido ruminal en un tubo de ensaye con 1 mL de ácido metafosfórico al 25% (v/v), para lograr una concentración de 4:1; las muestras se congelaron hasta su análisis.

Para determinar la concentración de AGV, las muestras se descongelaron y una alícuota de 1.5 mL se centrifugó a 12 000 rpm durante 10 minutos, el sobrenadante se colocó en viales de vidrio para cromatografía, se midió la concentración de AGV de acuerdo a lo propuesto por Erwin *et al.* (1961), utilizando 1 µL inyectado a un cromatógrafo de gases Perkin Elmer, modelo Claurus 500, con auto muestreador y equipado con una columna capilar FFAP con longitud de 15 m, temperatura del inyector de 240 °C, detector de ionización de flama (FID) de 250 C y de horno 140 °C con flujo de gases (H_2 y aire) de 40 mL min^{-1} para el aire y 400 mL min^{-1} para el hidrógeno.

La concentración de N-NH_3 se determinó de acuerdo a McCollough (1967). Una muestra del líquido ruminal descongelada de 2 mL se centrifugó a 3,000 rpm por 10 min, del sobrenadante se colectaron 20 µL y se depositaron en tubos de ensaye de 10 mL adicionando 1 mL de fenol y 1 mL de hipoclorito de sodio. Las muestras se incubaron en baño maría a 37 °C por 30 min y se adicionaron 5 mL de agua destilada para diluir las muestras, se agitaron en un vortex (Genie 2, modelo G-560) y se realizó la lectura en un espectrofotómetro de luz ultravioleta visible CARY 1-E VARIAN a una λ de 630 nm.

Degradación de MS *in situ*: Se incubaron en rumen 0.5 g de muestras del alimento en bolsas de nylon para digestibilidad *in situ* durante 0, 4, 8, 24, 48, 72 y 96 h en los días 19, 20 y 21 del periodo. La degradación de materia seca se tomó como porcentaje del peso de la muestra incubada en relación a la muestra degradada al finalizar los tiempos de incubación.

Componentes sanguíneos: el día 21 de cada periodo se tomaron muestras de sangre por punción de vena yugular, en tubos vacutainer® sin anticoagulante, las muestras fueron centrifugadas a 5000 rpm por 10 min a 4 ºC. Se separó y conservó el sobrenadante en congelación hasta su análisis.
Los metabolitos sanguíneos: urea, glucosa y triglicéridos, fueron determinados utilizando los kits comerciales: Urea FS, Glucose Gluc-DH FS y Triglycerides FS 5'; en un analizador químico Selectra Junior® Vital Scientific.

Análisis de laboratorio

En muestras de la dieta y heces fue determinada la materia seca (MS), materia orgánica (MO), nitrógeno (N) y cenizas (AOAC, 2003), fibra detergente neutro (FDN) y ácida; (FDA) se determinaron con el analizador de fibras ANKOM$^{200/220}$ de acuerdo al método descrito por Van Soest *et al.*, (1991).

Análisis estadístico

Se utilizó el diseño de cuadro latino 4 x 4, donde se evaluó el efecto de la dosis de enzima. Los datos fueron analizados con el procedimiento GLM (SAS, 2002) y comparación de medias con la prueba de Tukey (P≤0.05) (Steel y Torrie, 1996). El modelo estadístico fue el siguiente;

$$Y_{ijk} = \mu + H_i + C_{j+} t_{(k)} + \varepsilon_{ijk} \qquad i=1, 2\ldots t \;\; j= 1, 2, t \; k=1, 2, t$$

Donde,

Y_{ijk} = variable de respuesta en el periodo $_i$, animal $_j$, tratamiento $_k$.

μ = media general

S_i = efecto del periodo $_i$

H_j = efecto del animal $_j$

T_k = efecto del tratamiento $_k$

ε_{ijk} = error aleatorio

6.2 Influencia de celulasa o xilanasa en la producción de gas *in vitro* rumen y la fermentación ruminal del rastrojo de maíz

Animales

Como donador de inoculo ruminal se utilizó una vaca Pardo Suizo de 450 kg de PV, con fistula y cánula en rumen, alojada en un corral con agua y alimento *ad libitum,* que consistió en una dieta formulada para cubrir sus requerimientos (NRC, 2001), contenía heno de alfalfa y concentrado comercial (Purina®, Toluca, México) en una relación 1:1.

Sustrato

En el Estado de México se seleccionaron, al azar, tres lotes de rastrojo de maíz, de donde se recolectaron manualmente muestras de forraje. Las muestras fueron secadas a 65 ºC durante 48 h en una estufa de aire forzado, posteriormente homogenizadas y molidas (Molino Wiley®) a criba de 1 mm.

Tratamientos

Se evaluaron dosis crecientes (0, 10, 20, 40 y 80 µg/g MS) de enzimas exógenas celulasa (Cellulase® PLUS, Dyadic®) y xilanasa (Xylanase® PLUS, Dyadic®) en presentación líquida.

Recolección de inoculo ruminal

El inoculo ruminal se obtuvo, prepandial, directamente por extracción manual con un vaso de precipitados, el contenido ruminal se filtró a través de cuatro gasas y un colador de plástico, colocándose en un termo de plástico precalentado a 39 ºC para su transporte al laboratorio. En el laboratorio, el inoculo ruminal se mantuvo a una temperatura constante de 39 ºC y bajo flujo de CO_2.

Preparación de los viales de incubación

En viales de vidrio para antibiótico de 120 mL que contenían 0.5 g del sustrato, con la dosis y tipo de enzima, se añadieron 10 mL de líquido ruminal y 40 mL de solución buffer (Goering y Van Soest, 1970), sin adición de tripticasa, se mantuvo con flujo constante de CO_2 durante 30 seg., se colocaron tapones de neopreno y se sellaron con arillos de aluminio. Los viales fueron colocados en una incubadora (Riossa®) a 39 ºC durante 48 h.

Se incubaron 3 repeticiones por tratamiento (dosis de enzima). El experimento se repitió 3 veces (una corrida por semana).

Medición de gas

Las lecturas de producción de gas se realizaron a las 2, 4, 6, 8, 10, 12, 24, 36, 48 y 72 h de incubación. Para ello se utilizó la técnica del transductor de presión (Extech instruments, Waltham, USA) Theodorou *et al.,* (1994).

Detención de la fermentación

Transcurridas las 48 h de incubación, después de leer la producción de gas, los viales fueron sometidos a un shock térmico, para lo cual se colocaron en un congelador (-4 ºC) durante 5 min, esto con el fin de provocar la muerte de los microorganismos ruminales y detener el proceso de fermentación.

Medición de pH

Después de detener el proceso de fermentación, se destaparon los viales y fue medido y registrado el pH del medio.

Obtención del residuo

El contenido de los viales fue filtrado en crisoles de vidrio con filtro sinterizado (porosidad no.1, tamaño de poro de 100-160 µm, Pirex, Sotone, UK). Los crisoles con residuo fueron colocados en una estufa de aire forzado a 105 ºC durante una noche para estimar la desaparición de materia seca.

Análisis químicos

A una muestra de sustrato y a los residuos de la fermentación se les determinó el porcentaje de Materia Seca (% MS), Cenizas y Proteína total (%PC) (AOAC, 2003), Fibra Detergente Neutro (% FDN) y Fibra Detergente Ácido (FDA) (Van Soest *et al*., 1991), usando una unidad de analizador de fibra ANKOM200 (ANKOM Technology Corp., Macedon, NY, EE.UU.) sin usar alfa amilasa pero con sodio sulfito. Fue calculada la degradabilidad de la MS, DFDN y DFDA.

Cálculos

Para estimar la cinética de producción de gas (PG), se ajustaron los volúmenes de gas registrados (mL/g de MS) utilizando la opción NLIN de SAS (2002) según el modelo propuesto por France *et al*., (2000):

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

Dónde: *y* es el volumen de PG en el tiempo *t*; *b* es la PG asintótica (ml/g de MS); *c* es la velocidad fraccionaria de fermentación (/h), y *L* (h) es el tiempo de retardo antes de que se libere cualquier gas.

La energía metabolizable (EM, MJ/kg MS) y la digestibilidad *in vitro* de la materia orgánica (MO) fueron calculadas según Menke *et al.* (1979) como:

$$ME = 2.20 + 0.136\ GP\ (mL/0.5\ g\ de\ MS) + 0.057\ PC\ (g/kg\ de\ MS)$$

$$OMD = 148.8 + 8.89\ PG + 4.5\ PC\ (g/kg\ MS) + 0.651\ cenizas\ (g/kg\ MS)$$

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

Las concentraciones de ácidos grasos de cadena corta (AGV) se calcularon de acuerdo con Getachew *et al.* (2002) como:

$$\text{AGV (mmol/200 mg de MS)} = 0.0222\ \text{PG} - 0.00425$$

Dónde PG es la PG neta de 24 h (mL / 200 mg de MS).

Se calculó la producción de biomasa microbiana (PMC, por sus siglas en inglés) (Getachew *et al.,* 2002) como:

$$\text{Producción de PMC (mg/g de MS)} = \text{mg MSD} - (\text{mL de gas} \times 2.2\ \text{mg/mL})$$

Dónde, el 2.2 mg/mL es un factor estequiométrico que expresa los mg de C, H y O requeridos para la producción de AGV asociado a la producción de un mL de gas (Getachew *et al.*, 2002).

Diseño experimental

Para la PG *in vitro*, la degradabilidad y las variables de fermentación se utilizó un diseño completamente al azar considerando el tipo de enzima como factor fijo en el modelo lineal (Steel *et al.,* 1997) dentro de cada dosis enzimática. Los datos de cada una de las tres series dentro de la misma muestra se promediaron antes del análisis estadístico. Se utilizaron los valores medios de cada muestra individual como unidad experimental. El modelo estadístico fue:

$$Yijk = \mu + Zi + Dj + (Z \times D)\ ij + Eijk;$$

Donde Yijk es cada observación de la i-ésima enzima cuando se incuba en la j-ésima dosis; μ es la media general; Zi (i = 1-2) es el efecto enzimático; Dj es el efecto de la dosis enzimática (j = 1-5); (Z * D) ij es la interacción entre el tipo de enzima y la dosis de la enzima; Eijk es error experimental. Se utilizaron contrastes polinómicos lineales y cuadráticos para examinar las respuestas a los niveles crecientes de adición de las enzimas.

6.3 Producción de gas, metano, dióxido de carbono *in vitro* y cinética de fermentación de una ración mixta concentrada suplementada con xilanasa y *S. cerevisiae* incubada con líquido ruminal de cabras, borregos y novillos

Localización

La prueba se realizó en el Laboratorio de Nutrición Animal perteneciente al Programa de Ganadería del Colegio de Postgraduados Campus Montecillo, ubicado en el Km 36.5 de la carretera Los Reyes-Texcoco, Montecillo, Texcoco, Estado de México, a una altitud de 2250 msnm, el clima de la región es templado subhúmedo con lluvias en verano, época seca en invierno y una temperatura promedio anual de 15.2 °C. El experimento se realizó en los meses de junio a septiembre 2015.

Animales

Como donadores de inoculo ruminal se utilizaron 2 bovinos Holstein machos de 950 ± 20 kg de PV; 2 ovinos machos Rambouillet de 60 ± 2 kg de PV y 2 caprinos machos criollos de 50 ± 2 kg de PV; con fistula y cánula en rumen. Los animales fueron alojados en corrales por especie, con agua y alimento *ad libitum,* que consistió en raciones de heno de avena y concentrado en una relación 60:40, a las 08:00 y 16:00 h.

Sustrato

Se elaboró 1kg de una dieta que contenía: Grano de Sorgo Molido (52 %), Rastrojo de Maíz (30 %), Pasta de Soya (6 %), Melaza (8 %) y Urea (4 %). La dieta fue secada a 55 ºC durante 48 h, y posteriormente molida a criba de 1 mm.

A una muestra de sustrato se le determinó el porcentaje de Materia Seca (% MS), Cenizas y Proteína total (%PC) (AOAC, 2003), Fibra Detergente Neutro (% FDN) y Fibra Detergente Ácido (FDA) (Van Soest *et al*., 1991).

Solución stock

Se prepararon 4 soluciones stock que contenían: T1: agua destilada (100 mL); T2: 200 μL de Xylanase PLUS (Dyadic® proveniente de *Trichoderma reesei*) aforados a 100 mL; T3: 400 mg de *Saccharomyces cerevisae* (BIOCEL-F53) aforados a 100 mL y T4: 200 μL de Xylanase PLUS + 400 mg de *Saccharomyces cerevisae* aforados a 100 mL.

Recolección de inoculo ruminal

En bovinos, el inoculo ruminal se obtuvo directamente por extracción manual con un vaso de precipitados, el contenido ruminal se filtró a través de cuatro gasas y un colador de plástico, colocándose en un termo de plástico precalentado a 39 ºC para su transporte al laboratorio.

En ovinos y caprinos, el inoculo ruminal se obtuvo utilizando una sonda ruminal, conectada a un sistema de vacío, el inoculo ruminal se recuperó en un matraz Erlenmeyer de 500 mL, el contenido ruminal se filtró a través de cuatro gasas y un colador de plástico, colocándose en un termo de plástico precalentado a 39 ºC para su transporte al laboratorio. En el laboratorio, el inoculo ruminal se mantuvo a una temperatura constante de 39 ºC y bajo flujo de CO_2.

Preparación de soluciones para el medio de incubación

El medio de incubación consistió en una mezcla de solución buffer (Cuadro 9), solución macromineral (Cuadro 10), solución micromineral (Cuadro 11), solución reductora (Cuadro 12), resarzurina (Cuadro 13), líquido ruminal y agua destilada.

Cuadro 9. Solución Buffer

Bicarbonato de amonio (g)	NH_4HCO_3	4.0209
Bicarbonato de sodio (g)	$NaHCO_3$	35.1836

* Para preparar 1 L de solución

Cuadro 10. Solución Macromineral

Fosfato de sodio dibásico (g)	Na_2HPO_4	5.7299
Fosfato de potasio monobásico (g)	KH_2PO_4	6.2325
Sulfato de magnesio heptahidratado (g)	$MgSO_4 \cdot 7H_2O$	0.6032

* Para preparar 1 L de solución

Cuadro 11. Solución Micromineral

Cloruro de calcio dihidratado (g)	$CaCl_2 \cdot 2H_2O$	13.2
Cloruro de manganeso tetrahidratado (g)	$MnCl \cdot 4H_2O$	10.0
Cloruro de cobalto hexahidratado (g)	$CoCl \cdot 6H_2O$	1.0
Cloruro férrico (g)	$FeCl_3$	8.0

* Para preparar 100 mL de solución

Cuadro 12. Solución Reductora

Sulfato de sodio anhidro (g)	Na2SO4	0.57
Hidróxido de sodio 0.1 N (mL)	NaOH	4.00

* Para preparar 100 mL de solución

Cuadro 13. Resarzurina 1 %

Resarzurina (g)	1

* Para preparar 100 mL de solución

La solución buffer, solución macro y micro mineral, solución reductora y resarzurina, se prepararon un día antes de la incubación y se mantuvieron a 39 ºC dentro de una incubadora.

Preparación del medio de incubación

Previo a la incubación, fueron mezcladas las soluciones Buffer, macromineral, micromineral, resarzurina y agua destilada (Cuadro 14) en un matraz volumétrico, utilizando una platina y agitador magnético para mantener la temperatura y homogenizar la solución, cuando se estabilizó la temperatura en 39 ºC, se aplicó un flujo constante de CO_2, para posteriormente agregar el inoculo ruminal y la solución reductora (Cuadro 15).

Cuadro 14. Buffer para inoculo

	mL
S. Buffer	252.98
S. Macro mineral	252.98
S. Micro mineral	0.13
Resarzurina	1.30
Agua	505.95

*Para preparar 1013.33 mL de buffer

Cuadro 15. Medio de incubación

	mL
Buffer	666.67
Inoculo ruminal	333.33
S. Reductora	33.33

*Para preparar 1033.33 mL de medio

Preparación de viales con solución salina saturada

Se preparó una solución salina saturada con 400 g de NaCl en 1 L de agua destilada, con pH=2, se agregó como indicador 5 mL de naranja de metilo al 20%. La solución se depositó en viales serológicos de 60 mL, sin dejar espacio, se les colocaron tapones de neopreno y fueron sellados con arillos de aluminio. Se almacenaron protegidos de la luz, hasta su utilización.

Preparación de los viales de incubación

En viales de vidrio para antibiótico de 120 mL que contenían 0.5 g del sustrato, se les agregó 1 mL de solución stock y 40 mL del medio de incubación, se mantuvo con flujo constante de CO_2 durante 30 seg., se colocaron tapones de neopreno y se sellaron con arillos de aluminio. Los viales fueron colocados en una incubadora (Riossa®) a 39 ºC durante 48 h. Se incubaron 3 repeticiones por tratamiento (dosis de aditivo) en cada tipo de inoculo ruminal.

Medición de gas

Las lecturas de producción de gas se realizaron a las 2, 4, 6, 8, 10, 12, 24 y 48 h de incubación. Para ello se utilizó un aparato de desplazamiento de agua (Fedorak y Hrudey, 1983), el cual se diseñó con un soporte universal, un embudo cónico,

una bureta de 100 mL y dos mangueras de látex de 0.5 y 1 m de longitud y 3/8 de pulgada de diámetro. Los viales fueron punzados con una aguja de calibre 16 colocada en el extremo de la manguera. Se midió la producción de gas (mL) por el desplazamiento de agua en la bureta.

En la hora 48, se tomaron 5 ml de gas y se almacenaron en los viales con solución salina saturada (pH <2).

Detención de la fermentación

Transcurridas las 48 h de incubación, después de leer la producción de gas, los viales fueron sometidos a un shock térmico, para lo cual se colocaron en un congelador (-4 ºC) durante 5 min, esto con el fin de provocar la muerte de los microorganismos ruminales y detener el proceso de fermentación.

Toma de muestra

Fueron colectados:

- 4 mL del medio, el cual fue mezclado con 1 mL ácido metafosfórico al 25%, se agito levemente y la muestra se colocó en congelación hasta su análisis.
- 4 mL del medio, el cual fue mezclado con 1 mL formaldehído al 10% se agitó levemente y la muestra se colocó en refrigeración hasta su análisis.

Medición de pH

Después de detener el proceso de fermentación, se destaparon los viales y fue medido y registrado el pH del medio.

Obtención del residuo

El contenido de los viales fue filtrado en bolsas Ankom® Technologies F57 (a peso constante), con la ayuda de un sistema de filtrado conectado a una bomba de vacío, los viales fueron enjuagados con agua caliente en 3 ocasiones para

asegurar recuperar todo el residuo de la fermentación. Las bolsas fueron colocadas en una estufa de aire forzado a 55 ºC durante 48 h. La degradación de materia seca fue calculada considerando el peso inicial del sustrato y el peso del residuo.

Concentración de nitrógeno amoniacal

La concentración de nitrógeno amoniacal se realizó según lo estipulado por Broderick y Kang (1980). La muestra del medio acidificada se centrifugó a 3000 g x 10 min; 20 μL del sobrenadante mezclaron con 1 mL de fenol y 1mL de hipoclorito, la mezcla se incubó a 39 ºC por 30 min, posteriormente diluida con 5 mL de agua destilada. Las muestras fueron leídas en un espectrofotómetro de luz ultravioleta visible a 630 nm. La concentración en mg/dL resultante fue dividido entre 0.8 que es el factor de dilución del ácido metafosfórico al 25%.

Determinación de concentración de bacterias totales

La concentración de bacterias totales se determinó a las 48h de incubación, para ello se utilizó una cámara de Petroff-Hausser y un microscopio de contraste de fases (Carl Zeiss®) a una magnificación de 100x. Se tomaron 0.5 mL de la muestra del medio fijado con formaldehído al 10% y fueron diluidos en 4.5 mL de agua destilada. La concentración de bacterias por mL se determinó como el promedio de bacterias observado en cada cuadrícula, multiplicado por el factor de dilución y el factor de la cámara ($2x10^7$), de acuerdo con la siguiente fórmula:

$$Bacterias\ mL^{-1} = \bar{x} * FD1 * FD2 * 2^7$$

Dónde:

$\bar{x}$ = promedio de bacterias en cada cuadricula por tratamiento

FD1= primer factor de dilución (1.25)

FD2= segundo factor de dilución (10)

Determinación de concentración de protozoarios

Se obtuvieron 1 mL de la muestra fijada con formaldehído al 10% y fueron diluidos en 1 mL de agua destilada, se mezclaron y, con una pipeta de Pasteur fueron tomados 0.5 mL de la mezcla, los cuales fueron depositados en una cámara de Neubauer, posteriormente se observó en un microscopio de contraste Axiostar (Carl Zeiss®) a 400x magnificaciones. El conteo de protozoarios se realizó en ocho cuadrantes (4 de cada cuadrícula), tomándose como protozoarios viables aquellos que mantuvieron su integridad morfológica. La concentración de protozoarios por mL de medio de cultivo se estimó como el promedio de protozoarios observado en cada cuadricula, multiplicado por el factor de dilución y el factor de la cámara ($1x10^4$), de acuerdo con la siguiente fórmula:

$$Protozoarios\ mL^{-1} = \bar{x} * FD1 * FD2 * 10^4$$

Dónde:

$\bar{x}$ = promedio de protozoarios en ocho cuadrantes de la cámara de Neubauer

FD1= valor inverso de la dilución utilizada (5)

FD2= segundo valor inverso de la dilución utilizada (3)

Determinación de metano y CO_2

De los viales con solución salina saturada se tomó una muestra de 10 μL de la fase gaseosa y se inyectó en un cromatógrafo de gases PerkinElmer, Claurus 500. Se usó un detector de conductividad térmica (TCD), las temperaturas del horno, columna y TCD fueron de 80, 170 y 130°C respectivamente. El gas helio fue usado como gas acarreador. Los tiempos de retención fueron 1.74 y 2.04 min para CH_4 y CO_2, respectivamente.

Cálculos

Para estimar la cinética de producción de gas (PG), se ajustaron los volúmenes de gas registrados (mL/g de MS) utilizando la opción NLIN de SAS (2002) según el modelo propuesto por France *et al.*, (2000):

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

Dónde: *y* es el volumen de PG en el tiempo *t*; *b* es la PG asintótica (ml/g de MS); *c* es la velocidad fraccionaria de fermentación (/h), y *L* (h) es el tiempo de retardo antes de que se libere cualquier gas.

La energía metabolizable (EM, MJ/kg MS) y la digestibilidad *in vitro* de la materia orgánica (MO) fueron calculadas según Menke *et al.* (1979) como:

ME = 2.20 + 0.136 GP (mL/0.5 g de MS) + 0.057 PC (g/kg de MS)

OMD = 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.

Las concentraciones de ácidos grasos de cadena corta (AGV) se calcularon de acuerdo con Getachew *et al.* (2002) como:

AGV (mmol/200 mg de MS) = 0.0222 PG – 0.00425

Dónde PG es la PG neta de 24 h (mL / 200 mg de MS).

Se calculó la producción de biomasa microbiana (PMC, por sus siglas en inglés) (Getachew *et al.,* 2002) como:

Producción de PMC (mg/g de MS) = mg MSD - (mL de gas x 2.2 mg/mL)

Dónde, el 2.2 mg/mL es un factor estequiométrico que expresa los mg de C, H y O requeridos para la producción de AGV asociado a la producción de un mL de gas (Getachew *et al.*, 2002).

Diseño experimental

Se usó un diseño factorial 3 x 4 con 3 repeticiones. Los datos fueron analizados con el procedimiento GLM (SAS, 2002) y comparación de medias con la prueba de Tukey ($P \leq 0.05$) (Steel y Torrie, 1996). El modelo estadístico fue el siguiente;

$$Y_{ijk} = \mu + A_i + \beta_j + (A\beta)_{ij} + \varepsilon_{ijk} \qquad i=1,2\ldots t \;\; j= 1, 2, \ldots, t$$

Donde,

Y_{ijk} = es cada observación $_k$ del aditivo $_i$ cuando se incuba en el inoculo $_j$

μ = media general

A_i = efecto del aditivo $_i$

β_j = efecto del inoculo $_j$

T_k = efecto del tratamiento $_k$

ε_{ijk} = error aleatorio

VII. RESULTADOS

7.1 Efecto de la suplementación con xilanasa sobre el consumo de alimento, digestibilidad y fermentación ruminal de borregos Rambouillet.

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ANIMAL RESEARCH PAPER

Effects of xylanase supplementation on feed intake, digestibility and ruminal fermentation in Rambouillet sheep

L. H. VALLEJO[1], A. Z. M. SALEM[1]*, L. M. CAMACHO[2], A. M. KHOLIF[3], M. D. MARIEZCURRENA[4], M. CIPRIANO[2], M. U. ALONSO[1], J. OLIVARES[2] AND S. LOPEZ[5]

[1] *Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, México*
[2] *Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Guerrero, Cd. Altamirano-Iguala, Guerrero, México*
[3] *Dairy Science Department, National Research Centre, 33 Bohouth St. Dokki, Giza, Egypt*
[4] *Facultad de Ciencias Agrícola, Universidad Autónoma del Estado de México, Toluca, México*
[5] *Instituto de Ganadería de Montaña (IGM) CSIC-Universidad de León, Departamento de Producción Animal, Universidad de León, E-24071 León, Spain*

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SUMMARY

The present study aimed to investigate the effects of adding xylanase enzyme (XY) to a basal diet containing 300 g maize stover and 700 g concentrate/kg dry matter (DM) on feed intake, ruminal fermentation, total tract and ruminal digestibility, as well as some blood parameters. Four male Rambouillet sheep (39 ± 1·8 kg body weight), with permanent rumen and duodenum cannulae were used in a 4 × 4 Latin square design. Sheep were fed a basal diet without xylanase addition (control, XY0), or with the addition of xylanase at 1 (XY1), 3 (XY3) or 6 (XY6) µl/g of diet DM for 84 days, with four 21-day experimental periods. Feed intake, digestibility and rumen fermentation parameters were determined on days 16–21 in each experimental period, and the apparent ruminal neutral detergent fibre (NDF) digestibility was determined on days 16 and 17. Treatments XY1 and XY3 increased feed intake, whereas digestibility was increased with XY6. Ruminal NDF digestibility increased when sheep were fed diets treated with xylanase. Ruminal pH, ammonia-N and acetic acid increased with xylanase treated diets. Propionic acid concentration increased with diet XY1 at 3 h post-feeding, but after 9 h post-feeding its concentration decreased in the rumen of sheep fed xylanase treated diets. Xylanase had no effect on blood urea, phosphorus and triglycerides. Addition of xylanase at 6 µl/g DM in a diet containing 300 g maize stover and 700 g concentrate/kg DM and fed to Rambouillet sheep improved feed digestibility and ruminal fermentation without affecting blood parameters.

INTRODUCTION

Improving feed utilization in ruminant nutrition is one of the most important features that determine farming profitability. Many strategies have been considered to improve feed utilization; including, for example, the use of live yeast (Elghandour *et al*. 2014), phytogenic extracts (Salem *et al*. 2014) or fibrolytic enzymes (Valdes *et al*. 2015).

A large number of commercial enzyme products, either from fungal or bacterial sources, in relatively concentrated and purified forms and containing specific controlled enzyme activities, have been used in livestock feeding (Dean *et al*. 2013; Abdel-Aziz *et al*. 2015). Feeding fibre-degrading enzymes seems to improve feed utilization as well as animal performance (Khattab *et al*. 2011; Alsersy *et al*. 2015), but the mode of action remains unclear. Some possible modes of action have been postulated including hydrolysis of dietary fibre before ingestion, synergistic interaction with endogenous microbial enzymes within the rumen (Morgavi *et al*. 2004), favoured ruminal fermentation (Salem *et al*. 2013; Rojo *et al*. 2015), and enhanced ruminal microorganisms attachment and colonization to the plant cell wall (Wang *et al*. 2001).

* To whom all correspondence should be addressed. Email: asalem70@yahoo.com

Although some of the reported results on supplementing animal rations with fibrolytic enzymes are encouraging, they are also inconsistent. In some studies, exogenous enzymes improved feeding value and animal performance by enhancing fibre degradation, increasing intake and feed digestion *in vitro* (Salem *et al*. 2015*a*), *in situ* (Togtokhbayar *et al*. 2015) and *in vivo* (Salem *et al*. 2015*b*; Morsy *et al*. 2016). However, in other studies no effects of exogenous enzymes on feed intake and digestion were observed (Elwakeel *et al*. 2007; Dean *et al*. 2013). Although the reasons for this discrepancy are unknown, it could be due to differences in enzyme activity, application rate and composition, type of diet fed to the animals, physiological stage of the animal, time of enzyme delivery, ruminal activity and enzyme stability, enzyme-feed specificity and the portion of the diet to which enzymes are applied (Dean *et al*. 2013).

Exogenous enzyme may affect some serum metabolites that reflect the nutritional and health status of animals (Morsy *et al*. 2016). Xylanase is an exogenous enzyme that may alter ruminal degradation of feeds and change concentrations of fermentation end-products (Lin *et al*. 1995). Moreover, it may also cause an indirect glucose sparing effect through the pentose-phosphate pathway (Jackson & Nicolson 2002).

The objective of the present study was to investigate effects of adding an exogenous xylanase enzyme at different application rates on feed intake, ruminal fermentation, total tract and ruminal digestibility, and blood urea, phosphorus and triglyceride concentrations in Rambouillet sheep fed a basal diet with 300 g maize stover and 700 g concentrate/kg dry matter (DM).

MATERIALS AND METHODS

All procedures involved in handling animals during the experimental period were conducted according to the official Mexican standard of animal care (NOM-051-ZOO-1995).

Study location

The experiment was conducted at the animal metabolic unit and the laboratory of animal nutrition of the Colegio de Postgraduados, Texcoco, Montecillo, Estado de México, México (2240 m a.s.l.). The climate is moderately humid with an average temperature of 15–18 °C and annual rainfall of 650 mm. The experiment was conducted during the autumn.

Table 1. *Ingredients and chemical composition of the basal diet fed to Rambouillet sheep (g/kg dry matter (DM), unless otherwise stated)*

	g/kg DM
Ingredients	
Ground sorghum grain	520
Maize stover	300
Molasses	80
Soybean meal	60
Urea	40
Chemical composition	
Dry matter (g/kg fresh matter)	870
Organic matter	950
Crude protein	154
Ether extract	57
Neutral detergent fibre	448
Acid detergent fibre	252
Phosphorus	4·3
Calcium	2·5

Enzyme activity

The enzyme product (Xylanase® plus, Dyadic® PLUS, Dyadic International, Inc., Jupiter, FL, USA) was assessed for endoglucanase and xylanase activities as described by Robyt & Whelan (1972) by catalytic hydrolysis of xylan from oat spelt and determining the released reducing groups using alkaline copper reagent. The product contained 34 000–41 000 units of xylanase/g, 12 000–15 000 units of β-glucanase/g and 45 000–55 000 units of cellulase/g.

Animals, housing and feeding

Four Rambouillet rams, weighing 39 ± 1·8 kg body weight (BW) and fitted with permanent cannulae in the rumen (2·5 cm internal diameter (i.d.)) and duodenum (T-type 0·8 cm i.d.) were used. The sheep were housed in individual cages equipped with high flow valve steel water bowls and fed a basal diet composed of 300 g maize stover and 700 g concentrate/kg DM (Table 1) *ad libitum* for 84 days. The basal diet was balanced for minerals and vitamins and formulated to match the nutrient requirements of sheep according to NRC (1985) recommendations plus a margin of 0·10. The ingredients and chemical composition of the basal diet are shown in Table 1. At the beginning of the experiment, sheep were treated with Ivermectin (Ivomec®-F-1 1 ml/50 kg BW, subcutaneous), Bacterin (Covexin® 10 ml/animal; intramuscular) and vitamins A, D and E (Vigantol® ADE 1 ml/animal, intramuscular).

The experiment was laid out according to a 4 × 4 Latin square design with four treatments, i.e. four application rates of xylanase (XY), namely 0 (control, XY0), 1 (XY1), 3 (XY3) and 6 (XY6) µl/g DM of the basal diet. In the first experimental period, treatments were assigned randomly to the experimental units (sheep). Experimental periods consisted of 21 days with days 1–15 considered as the adaptation period to the experimental diets and days 16–21 as the measurement and sample collection period. Sheep were fed twice daily in two equal meals at 07·00 and 19·00 h. The enzyme was added at the corresponding application rate, mixed with the diet individually and fed at 07·00 h. During the collection period, i.e. days 16–21, the amount of feed offered was recorded and orts collected and weighed for determination of daily feed intake. Additionally, feeds were sampled daily, composited weekly, dried at 60 °C to constant weight and stored for later chemical analysis.

Feed digestibility

Total tract digestibility was determined by total faecal collection during days 16–21 of each period. Faeces were collected daily before the morning feeding and stored at −10 °C for later analysis. A sub-sample of about 100 g/kg of the total faeces collected from each sheep was taken daily and composited for chemical analysis.

Apparent ruminal fibre digestibility was determined on days 16 and 17 following the procedure of Kozloski *et al*. (2014). Duodenal digesta samples (approximately 50 ml) were collected from each sheep 4 h after morning feeding and then at 4 h intervals over a period of 48 h. Samples were obtained from the duodenal cannula, collected in a 100 ml amber vial and immediately frozen until analysis. Samples were subsequently thawed and dried at 55 °C for 48 h, homogenized and analysed for neutral detergent fibre (NDF) and acid detergent fibre (ADF).

Dried feed, feed orts and faecal samples were ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) using a 1 mm screen and analysed according to AOAC (1997) for DM (#930·15), ash (#942·05), ether extract (EE; #920·39), nitrogen (N; #954·01) and ADF (#973·18), while NDF was analysed according to Van Soest *et al*. (1991). Organic matter (OM, g/kg DM) content was calculated by difference (1000-g ash/kg DM).

Rumen and blood sampling and analysis

Rumen fluid was collected on day 18 of each experimental period, directly through the rumen cannula from the ventral sac of each sheep at 3, 6 and 9 h after morning feeding. The rumen samples (approximately 50 ml/sheep) were filtered immediately through four layers of cheesecloth, strained and stored in 45 ml glass bottles. Ruminal fluid pH was then determined using a portable pH meter (Orion, model SA 210, USA). Subsequently, 4 ml of rumen fluid was mixed with 1 ml of a solution of metaphosphoric acid (250 g/l) in a test tube and stored at −18 °C for subsequent volatile fatty acid (VFA) analysis. A sub-sample of 5 ml of rumen fluid was acidified with 5 ml of 0·2 M hydrochloric acid (HCl) for ammonia-N analysis.

Concentrations of acetic, propionic and butyric acids in rumen fluid were measured by gas-liquid chromatography (Hewlett Packard, Little Falls, DE, USA) using a capillary column (30 m length, 0·32 mm i.d., 0·25 mm film thickness; Elite-FFAP, Perkin Elmer Instruments, Shelton, WA, USA) according to the method of Erwin *et al*. (1961). The injector temperature was set at 240 °C, flame ionization detector at 250 °C and oven at 140 °C with hydrogen gas (H_2) and air flows at 40 and 400 ml/min, respectively.

Concentrations of ammonia-N were determined photometrically in an ultraviolet light spectrophotometer (VARIAN CARY 1-E, CA, USA) set at a wavelength of 630 nm according to McCullough (1967).

On day 21 of each experimental period and 4 h after morning feeding, a sample of 10 ml of blood was collected via jugular vein of each sheep into clean dry test tubes, without anticoagulant. Blood samples were centrifuged at 5000 ***g*** at 4 °C for 20 min. Serum was separated into 2 ml Eppendorf tubes and frozen at −20 °C until analysis. Blood serum samples were analysed for concentrations of urea, phosphorus and triglycerides using specific kits (Stanbio Laboratory, Boerne, TX, USA) according to the manufacturer's specifications.

Statistical analysis

Data on feed intake, digestibility, ruminal fermentation parameters (at each time post-feeding) and blood parameters were examined by analysis of variance according to a 4 × 4 Latin square design with four periods and four experimental diets (XY0, XY1, XY3, XY6) using PROC MIXED of SAS (SAS Institute 2006). One ram was used within each period and treatment. The statistical model was:

$$Y_{ijkl} = m + A_i + P_j + T_k + \varepsilon_{ijkl}$$

where Y_{ijkl} is the observation for a given response variable, μ is the overall mean, A_i is the random effect of

Table 2. *Feed intake, digestibility and blood metabolites in Rambouillet sheep fed a diet treated with increasing concentrations of xylanase (XY)*

	Diets*					*P* value				
	XY0	XY1	XY3	XY6	S.E.M. ($n = 4$)	Treatment effect	Control *v.* enzyme	Linear	Quadratic	Cubic
Feed intake (g DM/d)	1146	1211	1180	1004	56·2	0·043	0·034	0·066	0·508	0·684
Total tract digestibility (g digested/g ingested)										
Dry matter	0·58	0·60	0·57	0·75	0·041	0·047	0·027	0·021	0·014	0·901
Organic matter	0·59	0·62	0·59	0·77	0·039	0·032	0·019	0·018	0·014	1·000
Crude protein	0·54	0·56	0·51	0·71	0·053	0·036	0·044	0·069	0·015	0·702
Neutral detergent fibre	0·56	0·59	0·61	0·76	0·039	0·037	0·077	0·008	0·078	0·078
Acid detergent fibre	0·46	0·50	0·53	0·70	0·051	0·006	0·020	0·031	0·243	0·305
Apparent ruminal digestibility (g digested/g ingested)										
Neutral detergent fibre	0·36	0·39	0·42	0·46	0·024	0·045	0·036	0·122	0·078	0·078
Acid detergent fibre	0·29	0·31	0·33	0·34	0·022	0·055	0·584	0·294	0·050	0·305
Blood metabolites (mg/dl)										
Urea	44	49	50	49	4·4	0·358	0·643	0·346	0·147	0·355
Phosphorus	0·23	0·29	0·27	0·22	0·098	0·574	0·431	0·233	0·699	0·808
Triglycerides	8	10	6	9	2·0	0·511	0·945	0·917	0·339	0·369

DM, dry matter. *Diet (Table 1) without addition of xylanase (XY0) or with addition of xylanase at 1 (XY1), 3 (XY3) and 6 (XY6) µl/g DM.

ram, P_j is the fixed effect of period, T_k is the fixed effect of rate of addition of enzyme (XY0, XY1, XY3, XY6) and ε_{ijkl} is the residual error. Tukey's test was used for multiple comparisons of means. Polynomial contrasts (linear, quadratic and cubic effects) were fitted to the four rates of addition of the enzyme. A treatment (average of all treatments receiving XY) *v.* control contrast was also performed. Significance was declared at a level of $P < 0{\cdot}05$ and $P \leqslant 0{\cdot}10$ was considered as a tendency approaching significance.

RESULTS

Feed intake, digestibility and blood parameters

Sheep fed XY1 and XY3 had greater ($P = 0{\cdot}035$) feed DM intake than the control sheep (increases of 6 and 3% with XY1 and XY3, respectively). However, at the greatest application rate (XY6) feed intake was decreased slightly when compared with the control.

Sheep fed XY1 and XY6 had greater ($P < 0{\cdot}05$) total tract DM, OM and crude protein (CP) digestibility than the control sheep. Dry matter digestibility increased by 30% with XY6 when compared with the control diet. Digestibility of ADF increased linearly ($P = 0{\cdot}008$) with increasing enzyme application rates. The NDF digestibility of the enzyme treated diets tended ($P = 0{\cdot}077$) to be greater than that of the control sheep (Table 2).

Sheep fed enzyme had greater ($P < 0{\cdot}036$) ruminal NDF digestibility than control sheep. With XY6, ruminal NDF digestibility was increased by 28% when compared with the control diet. There was no difference in ruminal ADF digestibility between sheep fed the enzyme and the control diet (Table 2).

There were no differences in the blood concentrations of urea, phosphorus or triglycerides due to the different levels of xylanase addition (Table 2).

Ruminal fermentation

Ruminal pH of sheep fed with the enzyme was greater at all sampling times (*P* values were 0·050, 0·020 and 0·033 at 3, 6 and 9 h post-feeding, respectively) compared with control sheep. Within enzyme treatment, at the 3 h sampling, increasing enzyme application rate had no effect on pH. Sheep fed XY1 had maximum pH at the 6 h sampling and minimum pH at the 9 h sampling.

At the 6 h sampling, the ruminal ammonia-N concentration of sheep fed enzyme-treated diets was greater ($P = 0{\cdot}048$) than the control sheep. Within enzyme treatment, XY6 showed the maximum ammonia concentrations (linear effect, $P = 0{\cdot}028$), with an increase of 90% over the control value. At the 3 and 9 h sampling, there were no differences.

Table 3. *Rumen fermentation at different times post-feeding of a diet treated with increasing concentrations of xylanase (XY) in Rambouillet sheep*

	Diets*					*P* value			
	XY0	XY1	XY3	XY6	S.E.M. ($n = 4$)	Treatment effect	Control *v.* enzyme	Linear	Quadratic
pH									
3 h	6·0	6·3	6·2	5·8	0·17	0·031	0·050	0·198	0·385
6 h	5·8	6·1	6·0	6·1	0·13	0·057	0·020	0·041	0·051
9 h	5·8	5·9	6·0	6·0	0·06	0·116	0·033	0·039	0·236
Ammonia-N (mmol/l)									
3 h	17·3	15·9	15·5	16·6	3·11	0·976	0·730	0·933	0·690
6 h	7·7	9·0	12·7	14·6	1·86	0·012	0·048	0·028	0·929
9 h	8·6	8·3	8·2	8·2	0·43	0·906	0·517	0·604	0·650
Acetic acid (mmol/l)									
3 h	44·7	47·5	51·9	48·0	3·32	0·054	0·029	0·889	0·058
6 h	46·0	45·2	51·3	59·3	4·39	0·019	0·029	0·105	0·330
9 h	46·0	45·3	45·9	45·2	1·45	0·967	0·740	0·763	0·948
Propionic acid (mmol/l)									
3 h	43·2	47·3	45·0	42·3	1·91	0·035	0·047	0·379	0·026
6 h	42·7	42·9	39·1	45·7	2·75	0·047	0·046	0·530	0·213
9 h	47·5	45·7	44·2	45·9	2·19	0·047	0·041	0·672	0·037
Butyric acid (mmol/l)									
3 h	13·3	13·7	13·2	13·3	0·19	0·340	0·631	0·584	0·871
6 h	12·6	12·9	13·1	13·1	0·41	0·078	0·374	0·426	0·571
9 h	13·3	12·0	12·9	12·9	0·40	0·228	0·175	0·842	0·372

*Diet (Table 1) without addition of xylanase (XY0) or with addition of xylanase at 1 (XY1), 3 (XY3) and 6 (XY6) µl/g DM.

Acetic acid concentrations (mmol/l) were greater (quadratic effect, $P = 0·029$) in enzyme treatments compared with the control at 3 and 6 h post-feeding. With XY6 these concentrations were increased by 7 and 28% compared with the control at 3 and 6 h post-feeding, respectively. At 9 h, there were no significant differences. Propionic acid concentrations (mmol/l) at 3 h post-feeding were greatest in XY1 (quadratic effect, $P = 0·026$); however, at the 9 h sampling, all enzyme treatments had lower ($P = 0·041$) propionic acid concentrations compared with the control. Among enzyme application rates, XY3 had the lowest propionic acid concentration (quadratic effect, $P = 0·037$). No effects were observed on ruminal butyric acid concentrations between different treatments at all sampling times (Table 3).

DISCUSSION

Feed intake

Addition of xylanase to diets at low (i.e. XY1) and moderate (i.e. XY3) rates increased feed intake by about 6 and 3%, respectively, compared with XY0; however, intake decreased by 9% when xylanase was applied at a greater concentration (i.e. XY6) compared with XY0. Therefore, addition of fibrolytic enzymes at certain concentrations may increase the intake of fibrous feeds. Beauchemin *et al.* (2003) concluded that high rates of enzyme application could be less effective than low rates of application in increasing feed intake, indicating the importance of determining the optimal rate of enzyme addition. The current results are in agreement with Gado *et al.* (2009), who observed about 13% greater DM intake in dairy cows due to enzyme supplementation at 40 g/day.

Digestibility

When xylanase was applied to the diet at the highest concentration (i.e. XY6), whole tract digestibility was increased (by 28–42%) compared with other xylanase concentrations. Improving digestibility, in particular, that of the fibre fractions is the main purpose of adding fibrolytic enzymes to ruminant feeds. Improved digestibility with xylanase at some

application rates supports the hypothesis that a suitable enzyme concentration could improve fermentation efficiency during the initial stages of digestion (Jalilvand *et al*. 2008).

The greater digestibility observed with the XY6 diet may be related, as previously mentioned, to improved rate of ruminal digestion of the potentially digestible NDF fraction (Yang *et al*. 1999) and to changes in gut viscosity (Hristov *et al*. 2000); although these features were not determined in the present study. Altered ruminal fermentation (Kholif & Aziz 2014; Rojo *et al*. 2015), enhanced microbial attachment and colonization to the plant cell wall (Wang *et al*. 2001) and complementary interactions with ruminal microbial enzymes (Morgavi *et al*. 2004) are different possible reasons for the improved rate of ruminal digestion.

In most reports, addition of fibrolytic enzymes to the ruminant feedstuffs increased the numbers of non-fibrolytic and fibrolytic bacteria in rumen fluid and provided more total polysaccharidase activity to digest feedstuffs (Giraldo *et al*. 2008). Mao *et al*. (2013) found that addition of cellulase and xylanase increased the numbers of total bacteria and *Fibrobacter succinogenes* in *in vitro* incubation medium resulting in enhanced fermentation. Results in the present study are consistent with Khattab *et al*. (2011) and Salem *et al*. (2013; 2015*b*), who observed greater feed digestibility in response to exogenous enzyme addition.

Ruminal NDF digestibility was increased by 7·5, 17 and 28% (compared with the control diet), respectively, with increasing xylanase application rates. Thus, the increased total tract fibre digestibility seems to be due, in part, to enhance fibre digestion in the rumen. Fibrolytic enzymes not only improve fibrolytic activity in the rumen, but also raise xylanase activity in the small intestine (Hristov *et al*. 1998, 2000). Hristov *et al*. (1998) reported that addition of enzymes elevated duodenal xylanase activity by 30% and cellulase activity by 2–5%. Hristov *et al*. (2000) showed that xylanase activity in the faeces was increased with enzyme supplementation, suggesting that xylanase and probably other exogenous fibrolytic enzymes, may work synergistically with the microbes within the large intestine.

Blood metabolites

None of the measured blood metabolites (urea, phosphorus and triglycerides) was affected by xylanase addition to feed and all were found within the reference ranges (Boyd 1984). Serum urea concentration is an indicator of the nutritional status of sheep (Kumar *et al*. 1981), in particular regarding the provision of total and degradable protein in the feed. Normal serum urea values indicate that protein catabolism was not increased in the muscles and that kidney function was not adversely affected by diet.

Ruminal fermentation

Sheep fed xylanase had greater ruminal pH values compared with the control. One of the most important factors affecting fibre digestion is ruminal pH. For xylanase treatments, rumen pH ranged from 5·98 to 6·15, which was within the range considered acceptable for fibre digestion (Ørskov & Ryle 1990). Fibrolytic bacteria are very sensitive to ruminal pH changes (Sung *et al*. 2007). Greater ruminal pH values are more favourable for fibrolytic microbial activity than low ruminal pH (Sung *et al*. 2007).

Ruminal ammonia-N concentrations ranged from 7·7 to 17·3 mmol/l which were above the range that Satter & Slyter (1974) considered as sufficient for microbial protein synthesis. Greater ruminal ammonia-N concentrations in sheep fed the enzyme treated diets (XY6 and XY3) compared with the un-supplemented control support the possibility that xylanase enhances rumen protein degradation, probably in response to a shift in ruminal microbiota (Salem *et al*. 2013). Kholif & Aziz (2014) found that feeding goats on diets treated with a fibrolytic enzyme elevated ruminal ammonia-N concentration compared with un-supplemented control diets. The observed dose-effects reinforce the importance of defining the optimum application rate of enzyme for better feed utilization.

Greater acetic acid concentrations were obtained with xylanase-treated diets (especially with XY6) compared with the control. Improving fibre digestion usually alters rumen fermentation and affects the production of individual VFA. The greater acetic acid concentrations with xylanase addition could be associated with improved digestion of structural carbohydrates (Soltan *et al*. 2013). Changes in individual VFA concentrations observed when fibrolytic enzymes were added to feed suggest that these exogenous enzymes could affect microbial growth and activity, causing a shift in the metabolic pathways by which specific microbes utilize substrates (Almaraz *et al*. 2010). Shifts in ruminal fermentation may be the result of altered fibre structure, which could stimulate microbial colonization (Giraldo *et al*. 2008), or a

shift in the species profile of fibre-colonizing bacteria in response to enzyme addition (Wang *et al*. 2001). Gado *et al*. (2009) and Salem *et al*. (2013) also observed greater acetic acid concentrations in the rumen when animals were fed diets supplemented with exogenous enzymes.

Among the tested xylanase application rates, concentrations of 3 and 6 µl xylanase/g DM of the basal diet resulted in enhanced digestibility and ruminal fermentation in Rambouillet sheep. However, with 6 µl xylanase/g DM of the basal diet feed intake decreased, whereas ruminal ammonia-N and individual VFA increased compared with the other rates of enzyme addition. Generally, addition of xylanase had no effects on blood serum concentrations of urea, phosphorus and triglycerides.

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REFERENCES

Abdel-Aziz, N. A., Salem, A. Z. M., El-Adawy, M. M., Camacho, L. M., Kholif, A. E., Elghandour, M. M. Y. & Borhami, B. E. (2015). Biological treatments as a mean to improve feed utilization in agriculture animals – an overview. *Journal of Integrative Agriculture* **14**, 534–543.

Almaraz, I., González, S. S., Pinos-Rodríguez, J. M. & Miranda, L. A. (2010). Effects of exogenous fibrolytic enzymes on *in sacco* and *in vitro* degradation of diets and on growth performance. *Italian Journal of Animal Science* **9**, 6–10.

Alsersy, H., Salem, A. Z. M., Borhami, B. E., Olivares, J., Gado, H. M., Mariezcurrena, M. D., Yacuot, M. H., Kholif, A. E., El-Adawy, M. & Hernandez, S. R. (2015). Effect of Mediterranean saltbush (*Atriplex halimus*) ensilaging with two developed enzyme cocktails on feed intake, nutrient digestibility and ruminal fermentation in sheep. *Animal Science Journal* **86**, 51–58.

AOAC (1997). *Official Methods of Analysis of the Association of Official Analytical Chemist*, Vol. **1**, 16th edn, Washington, DC: Association of Official Analytical Chemists.

Beauchemin, K. A., Colombatto, D., Morgavi, D. P. & Yang, Y. Z. (2003). Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Science* **81**, (E Suppl.) **2**, E37–E47.

Boyd, J. W. (1984). The interpretation of serum biochemistry test results in domestic animals. *Veterinary Clinical Pathology* **13**, 7–14.

Dean, D. B., Staples, C. R., Littell, R. C., Kim, S. C. & Adesogan, A. T. (2013). Effect of method of adding a fibrolytic enzyme to dairy cow diets on feed intake digestibility, milk production, ruminal fermentation, and blood metabolites. *Animal Nutrition and Feed Technology* **13**, 337–353.

Elghandour, M. M. Y., Vázquez Chagoyán, J. C., Salem, A. Z. M., Kholif, A. E., Martínez Castañeda, J. S., Camacho, L. M. & Cerrillo-Soto, M. A. (2014). Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on *in vitro* gas production kinetics and degradability of four fibrous feeds. *Italian Journal of Animal Science* **13**, 295–301.

Elwakeel, E. A., Titgemeyer, E. C., Johnson, B. J., Armendariz, C. K. & Shirley, J. E. (2007). Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. *Journal of Dairy Science* **90**, 5226–5236.

Erwin, E. S., Marco, G. J. & Emery, E. M. (1961). Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *Journal of Dairy Science* **44**, 1768–1771.

Gado, H. M., Salem, A. Z. M., Robinson, P. H. & Hassan, M. (2009). Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. *Animal Feed Science and Technology* **154**, 36–46.

Giraldo, L. A., Tejido, M. L., Ranilla, M. J., Ramos, S. & Carro, M. D. (2008). Influence of direct-fed fibrolytic enzymes on diet digestibility and ruminal activity in sheep fed a grass hay-based diet. *Journal of Animal Science* **86**, 1617–1623.

Hristov, A. N., McAllister, T. A. & Cheng, K.-J. (1998). Stability of exogenous polysaccharide-degrading enzymes in the rumen. *Animal Feed Science and Technology* **76**, 161–168.

Hristov, A. N., McAllister, T. A. & Cheng, K.-J. (2000). Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: effects on nutrient digestion in cattle fed a barley grain diet. *Journal of Animal Science* **78**, 477–487.

Jackson, S. & Nicolson, S. W. (2002). Xylose as a nectar sugar: from biochemistry to ecology. *Comparative Biochemistry and Physiology Part B. Biochemistry and Molecular Biology* **131**, 613–620.

Jalilvand, G., Odongo, N. E., Lopez, S., Naserian, A., Valizadeh, R., Shahrodi, E., Kebreab, E., France, J. (2008). Effects of different levels of an enzyme mixture on *in vitro* gas production parameters of contrasting forages. *Animal Feed Science and Technology* **146**, 289–301.

Khattab, H. M., Gado, H. M., Kholif, A. E., Mansour, A. M. & Kholif, A. M. (2011). The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. *International Journal of Dairy Science* **6**, 267–277.

Kholif, A. E., Gouda, G. A., Morsy, T. A., Salem, A. Z. M., Lopez, S. & Kholif, A. M. (2015). *Moringa oleifera* leaf meal as a protein source in lactating goat's diets: feed intake, digestibility, ruminal fermentation, milk yield and

composition, and its fatty acids profile. *Small Ruminant Research* **129**, 129–137.

Kholif, A. M. & Aziz, H. A. (2014). Influence of feeding cellulytic enzymes on performance, digestibility and ruminal fermentation in goats. *Animal Nutrition and Feed Technology* **14**, 121–136.

Kozloski, G. V., Stefanello, C. M., Mesquita, F. R., Alves, T. P., Ribeiro Filho, H. M. N., Almeida, J. G. R. & Moraes Genro, T. C. (2014). Technical note: evaluation of markers for estimating duodenal digesta flow and ruminal digestibility: acid detergent fiber, sulfuric acid detergent lignin, and n-alkanes. *Journal of Dairy Science* **97**, 1730–1735.

Kumar, N., Singh, U. B. & Verma, D. N. (1981). Effect of different levels of dietary protein and energy on growth of male buffalo calves. *Indian Journal of Animal Science* **51**, 513–517.

Lin, Y., Vonk, R. J., Slooff, M. J. H., Kuipers, F. & Smit, M. J. (1995). Differences in propionate-induced inhibition of cholesterol and triacylglycerol synthesis between human and rat hepatocytes in primary culture. *British Journal of Nutrition* **74**, 197–207.

Mao, H. L., Wu, C. H., Wang, J. K. & Liu, J. X. (2013). Synergistic effect of cellulase and xylanase on *in vitro* rumen fermentation and microbial population with rice straw as substrate. *Animal Nutrition and Feed Technology* **13**, 477–487.

McCullough, H. (1967). The determination of ammonia in whole blood by direct colorimetric method. *Clinica Chimica Acta* **17**, 297–304.

Morgavi, D. P., Beauchemin, K. A., Nsereko, V. L., Rode, L. M., McAllister, T. A. & Wang, Y. (2004). *Trichoderma* enzymes promote *Fibrobacter succinogenes* S85 adhesion to, and degradation of, complex substrates but not pure cellulose. *Journal of the Science of Food and Agriculture* **84**, 1083–1090.

Morsy, T. A., Kholif, A. E., Kholif, S. M., Kholif, A. M., Sun, X. & Salem, A. Z. M. (2016). Effects of two enzyme feed additives on digestion and milk production in lactating Egyptian buffaloes. *Annals of Animal Science* **16**, 209–222.

NRC (1985). *Nutrient Requirements of Sheep*, 6th edn, Washington, DC: National Academy Press.

Ørskov, E. R. & Ryle, R. (1990). *Energy Nutrition in Ruminants*. New York: Elsevier Science Publishers.

Robyt, J. F. & Whelan, W. J. (1972). Reducing value methods for maltodextrins. 1. Chain-length dependence of alkaline 3,5-dinitrosalicylate and chain length independence of alkaline copper. *Analytical Biochemistry* **45**, 510–516.

Rojo, R., Kholif, A. E., Salem, A. Z. M., Elghandour, M. M. Y., Odongo, N. E., Montes De Oca, R., Rivero, N. & Alonso, M. U. (2015). Influence of cellulase addition to dairy goat diets on digestion and fermentation, milk production and fatty acid content. *Journal of Agricultural Science, Cambridge* **153**, 1514–1523.

Salem, A. Z. M., Gado, H. M., Colombatto, D. & Elghandour, M. M. Y. (2013). Effects of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. *Livestock Science* **154**, 69–73.

Salem, A. Z. M., Kholif, A. E., Elghandour, M. M. Y., Buendía, G., Mariezcurrena, M. D., Hernandez, S. R. & Camacho, L. M. (2014). Influence of oral administration of *Salix babylonica* extract on milk production and composition in dairy cows. *Italian Journal of Animal Science* **13**, 10–14.

Salem, A. Z. M., Ammar, H., Kholif, A. E., Elghandour, M. M. Y. & Ortiz, L. B. (2015*a*). Effect of glucoamylase enzyme extract on *in vitro* gas production and degradability of two diets with 25% of corn or sorghum grains. *Indian Journal of Animal Science* **85**, 183–188.

Salem, A. Z. M., Alsersy, H., Camacho, L. M., El-Adawy, M. M., Elghandour, M. M. Y., Kholif, A. E., Rivero, N., Alonso, M. U. & Zaragoza, A. (2015*b*). Feed intake, nutrient digestibility, nitrogen utilization, and ruminal fermentation activities in sheep fed *Atriplex halimus* ensiled with three developed enzyme cocktails. *Czech Journal of Animal Science* **60**, 185–194.

SAS Institute (2006). *SAS 9·0 User's Guide: Statistics, version 9.0*. Cary, NC: SAS Institute.

Satter, L. D. & Slyter, L. L. (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro*. *British Journal of Nutrition* **32**, 199–208.

Soltan, Y. A., Abdalla, A. L., Silva, L. R. F., Natel, A. S., Morsy, A. S. & Louvandini, H. (2013). Response of different tropical pasture grass species to treatments with fibrolytic enzymes in terms of *in vitro* ruminal nutrient degradation and methanogenesis. *Animal Nutrition and Feed Technology* **13**, 551–568.

Sung, H. G., Kobayashi, Y., Chang, J., Ha, A., Hwang, I. H. & Ha, J. K. (2007). Low ruminal pH reduces dietary fiber digestion via reduced microbial attachment. *Asian-Australasian Journal of Animal Sciences* **20**, 200–207.

Togtokhbayar, N., Cerrillo, M. A., Rodríguez, G. B., Elghandour, M. M. M. Y., Salem, A. Z. M., Urankhaich, C., Jigjidpurev, S., Odongo, N. E. & Kholif, A. E. (2015). Effect of exogenous xylanase on rumen *in vitro* gas production and degradability of wheat straw. *Animal Science Journal* **86**, 765–771.

Valdes, K. I., Salem, A. Z. M., Lopez, S., Alonso, M. U., Rivero, N., Elghandour, M. M. Y., Domínguez, I. A., Ronquillo, M. G. & Kholif, A. E. (2015). Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. *Journal of Agricultural Science, Cambridge* **153**, 732–742.

Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre, and non-starch carbohydrates in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.

Wang, Y., McAllister, T. A., Rode, L. M., Beauchemin, K. A., Morgavi, D. P., Nsereko, V. L., Iwaasa, A. D. & Yang, W. (2001). Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the rumen simulation technique (Rusitec). *British Journal of Nutrition* **85**, 325–332.

Yang, W. Z., Beauchemin, K. A. & Rode, L. M. (1999). Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. *Journal of Dairy Science* **82**, 391–403.

7.2 Influencia de celulasa o xilanasa en la producción de gas *in vitro* rumen y la fermentación ruminal del rastrojo de maíz

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Influence of cellulase or xylanase on the *in vitro* rumen gas production and fermentation of corn stover

L H VALLEJO[1], A Z M SALEM[2], A E KHOLIF[3], M M Y ELGHANGOUR[4], R C FAJARDO[5], N RIVERO[6], A Z BASTIDA[7] and M D MARIEZCURRENA[8]

Universidad Autónoma del Estado de México, Estado de México, México

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ABSTRACT

In vitro gas production (GP) technique was used to investigate effect of exogenous enzymes cellulase (CEL) or xylanase (XYL) at different doses on *in vitro* fermentation characteristics of corn stover. Enzymes were supplemented at 0 (control), 10, 20, 40 and 80 µg/g DM. Gas production was determined at 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h of incubation. After 72 h, the incubation was stopped and supernatant pH was determined, and filtered to determine dry matter (DMD), neutral detergent fiber (NDFD) and acid detergent fiber (ADFD) degradabilities. Interaction effects occurred for enzyme type and dose for all measured gas parameters with exception of the lag time, DMD, organic matter degradability (OMD), NDFD, metabolizable energy (ME), short chain fatty acids (SCFA) and microbial crude protein (MCP) production. Cellulase and XYL increased GP ($P<0.05$) at different incubation hours with better results at the dose of 40 µg/g DM. The dose 80 µg XYL/g DM had the lowest GP compared to other doses. In addition, CEL and XYL decreased pH with increasing OMD, ME, SCFA and MCP production at 40 µg/g DM of corn stover. The present results suggested that the level of CEL and XYL at 40 µg/g DM have higher GP than other levels of enzymes, imply this level can be more effectively to improve rumen fermentation; however, the difference of XYL between treatments and control was less than that of CEL.

Key words: Cellulase, Corn straw, Fibrolytic enzyme, *In vitro* fermentation, Xylanase

Roughages are the main sources of feed for ruminants (Kholif *et al*. 2014). Fibrous feeds are characterized by low nutritive value. High fiber of fibrous feeds prevents the access of ruminal enzymes to the plant cell wall and reduce nutrient digestibility (Abdel-Aziz *et al*. 2015, Elghandour *et al*. 2015, Togtokhbayar*et al*. 2015). High fiber content and plant epidermal surface with a high concentration of silica prevents the access of ruminal enzymes to the plant cell wall and reduce nutrient digestibility (Khattab *et al*. 2013, Kholif *et al*. 2014). Corn stover is one of the agro-byproducts and can be used in ruminant feeding after upgrading its nutritive value (Elghandour *et al*. 2014). Hence, there is a need to develop feeding strategies that improve the nutritive value of such fibrous feeds. Using fibrolytic enzymes (e.g. cellulase, xylanase) for this purpose will prove effective.

Exogenous fibrolytic enzymes are used to improve carbohydrate and cell wall degradation of low quality feeds (Alsersy *et al*. 2015, Salem *et al*. 2015a,Valdes *et al*. 2015). The modes of actions proposed to explain the improved nutritive value of low quality feeds with high fiber contents are: enhanced attachment by rumen microorganisms (Nsereko *et al*. 2002), improving ruminal fermentation working synergetically with endogenous rumen microbial enzymes (Khattab *et al*. 2011), creation of a stable complexes between enzymes and feeds (Kung *et al*. 2000), and possible alteration in the fiber structure stimulating microbial colonization (Giraldo *et al*. 2004). The aim of the current experiment is to investigate the effect of adding increasing doses of cellulase (CEL) or xylanase (XYL) on *in vitro* fermentation kinetics and gas production (GP) of corn stover.

Present address: [1,2,4,5](asalem70@yahhoo.com), Facultad de Medicina Veterinaria y Zootecnia. [3]Dairy Science Department, National Research Centre, 33 Bohouth St. Dokki, Giza, Egypt. [6,7]Universidad Autónoma de Hidalgo, Rancho Universitario, Av. Universidad Km. 1, Ex-Hda. de Aquetzalpa AP 32, CP 43600, Tulancingo. Hidalgo, México. [8](nekkane16@hotmail.com), Facultad de Ciencias Agrícola, Universidad Autónoma del Estado de México, Toluca, México.

MATERIALS AND METHODS

Corn stover and enzyme products: Corn stover with chemical composition (g/kg DM): 959.7 organic matter (OM), 62.9 crude protein (CP), 476.7 neutral detergent fiber (NDF) and 274.4 acid detergent fiber (ADF) was used as incubation substrate. Corn stover samples were dried at 65 °C for 48 h in a forced air oven until constant weight and then ground in a Wiley mill to pass a 1 mm sieve and stored in plastic bags for subsequent determination of chemical composition and *in vitro* GP. Cellulase (CEL) and xylanase (XYL) enzymes in liquid form were tested at 0, 10, 20, 40

and 80 µg/g DM corn stover. A stock solution of each enzyme was prepared for each treatment (10, 20, 40 and 80 mg/L), so that the intended concentration in the fermentation cultures was achieved by dispensing 1 ml of each stock solution in each serum bottle topping the sample of feed. Activities of the CEL product were 30,000 to 36,000 units of cellulase/g and 7500 to 10,000 units of ß-glucanase/g while activities of XYL were 34,000 to 41,000 units of xylanase/g, from 12,000 to 15,000 units of beta-glucanase/g and 45,000 to 55,000 units of cellulase/g. Enzyme activities were provided by manufacturer.

In vitro *incubations:* Rumen inoculum was collected before morning feeding from a Brown Swiss cow (450 kg body weight) fitted with permanent rumen cannula and fed *ad lib.* a total mixed ration made up of 1:1 concentrate and alfalfa hay, formulated to cover its nutrient requirements (NRC 2001) with a full access to fresh water.

Obtained ruminal contents were flushed with CO_2, mixed and strained through 4 layers of cheese cloth into a flask with O_2-free headspace. Corn stover (1 g) was weighed into 120 ml serum bottles with appropriate addition of enzyme doses/g DM, applied immediately before incubation with rumen buffered solution. Consequently, 10 ml of particle free ruminal fluid was added to each bottle followed by 40 ml of the buffer solution of Goering and Van Soest (1970), with no trypticase added, in a 1:4 (v/v) proportion.

Bottles (90) were used during the incubation runs (2 enzymes × 5 doses × 3 replicates × 3 runs) plus 3 bottles as blanks (i.e., buffered rumen fluid only), were incubated for 72 h. After filling all bottles with substrates and inoculum medium, bottles were immediately closed with rubber stoppers, shaken and placed in the water-bath at 39 °C. The volumes of GP were recorded at times of 2, 4, 6, 8, 10, 12, 14, 24, 36, 48 and 72 h of incubation. The GP was recorded using the pressure transducer technique of Theodorou *et al.* (1994).

Nutrient degradability: At the end of incubation after 72 h, the fermentation process was stopped by swirling the bottles in ice, and the contents of each serum bottle were filtered under vacuum through glass crucibles with a sintered filter. The obtained fermentation residues were dried at 105ºC overnight to estimate DM disappearance. Both of NDF and ADF were determined in the residues after DM determinations and DM (DMD), NDF (NDFD) and ADF (ADFD) degradability were calculated. Blanks were used to correct for substrate contamination from enzyme or ruminal fluid.

Chemical analysis: Samples of the corn stover were analyzed for DM, ash and N according to AOAC (1997). The NDF and ADF content (Van Soest *et al.* 1991) of both feed and fermentation residues were determined using an fiber analyzer unit without use of an alpha amylase but with sodium sulphite. Both NDF and ADF are expressed without residual ash.

Calculations and statistical analysis: To estimate kinetic parameters of GP, gas volumes recorded (ml/g DM) were fitted using the NLIN option of SAS (2002) according to France *et al.* (2000) model as:

$$y = b \times (1–e^{-c(t–L)})$$

where, *y*, volume of GP at time t; *b*, asymptotic GP (ml/g DM); *c*, fractional rate of fermentation (/h); and *L* (h), discrete lag time prior to any gas is released. Metabolizable energy (ME; MJ/kg DM) and *in vitro* OM digestibility (OMD; g/kg OM) were estimated according to Menke *et al*. (1979).

Short chain fatty acid concentrations (SCFA) and microbial crude protein (MCP) productions were calculated according to Getachew *et al*. (2002).

The experimental design for the *in vitro* ruminal GP, degradability and fermentation parameters analysis was a completely random design considering enzyme type as fixed factors in the linear model within each enzyme dose using the PROC GLM option of SAS (2002). Data of each of the 3 runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample were used as the experimental unit. The statistical model was: $Y_{ijk} = \mu + Z_i + D_j + (Z \times D)_{ij} + E_{ijk}$; where $Y_{ijk,}$ every observation of the *I*th enzyme when incubated in the *j*th dose; µ, general mean; Z_i (i=1–2), enzyme effect; $D_{j,}$ enzyme dose effect (j=1–5); $(Z \times D)_{ij}$, interaction between enzyme type and enzyme dose; and E_{ijk}, experimental error. Linear and quadratic polynomial contrasts were used to examine responses to increasing addition levels of the enzymes.

RESULTS AND DISCUSSION

In vitro *gas production:* With exception of the fraction *L*, interaction effects occurred (P<0.05) for enzyme type × enzyme dose, revealing that GP is enzyme preparation and enzyme dose dependent. Enzyme dose had linear effect (P<0.05) on GP at different incubation hours with quadratic effect (P=0.041) on the fraction *c* (Table 1). Addition of CEL or XYL to corn stover increased GP (P<0.05) with greater (P<0.05) effect at the dose 40 µg/g DM. The effectiveness of enzymes depends upon substrate, enzyme specificity and enzyme dose causing variable responses with different enzyme preparations and doses (Salem *et al*. 2015b). This supports the hypothesis that a suitable enzyme dose could improve fermentation efficiency. Increased GP indicated the increased fermentable material with enzyme addition (Elghandour *et al*. 2015). Increased GP without affecting lag time could be due to release of polysaccharidase from corn stover, which provided fermentable carbohydrate to stimulate microbial growth. Another possible reason is the increased numbers of fibrolytic and nonfibrolytic bacteria in the rumen, which is very clear in the current study as the MCP production increased with enzyme addition (Nsereko *et al*. 2002). Mao *et al*. (2013) observed that addition of CEL and XYL increased numbers of total bacteria and *Fibrobacte rsuccinogens* in the incubation medium with improving *in vitro* fermentation.

The dose 80 µg XYL/g DM had the lowest GP (P<0.05) compared to other doses (Table 1); however, the higher dose

Table 1. Impact of cellulase (CEL) and xylanase (XYL) at different doses on *in vitro* gas production of corn stover at different hours of incubation

Enzyme	Dose (µg/g DM)	GP parameters			*In vitro* GP, 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	36 h	48 h	72 h
CEL	0	213.2	0.074	3.69a	11.3c	23.8c	42.4c	65.0c	91.5c	117.9b	175.9b	203.9b	218.0b	225.7b
	10	231.5	0.070	3.28ab	15.3b	30.4b	51.2bc	74.7bc	102.1bc	129.5ab	190.7ab	221.1ab	236.8ab	246ab
	20	227.2	0.073	3.23ab	16.0b	31.9b	53.6ab	77.8ab	104.9abc	131.6ab	191.7ab	220.3ab	234.9ab	242.8ab
	40	231.1	0.080	3.13ab	19.4a	37.9a	62.3a	88.7a	117.5a	144.9a	203.9a	230.7a	244.5a	251.5a
	80	230.7	0.074	2.61b	19.4a	37.6a	60.7ab	85.3ab	112.1ab	138.1a	196.4a	223.1ab	237.4ab	245.2ab
P value	Linear	0.067	0.939	0.055	0.001	0.001	0.003	0.005	0.013	0.022	0.030	0.029	0.030	0.034
	Quadratic	0.087	0.242	0.508	0.079	0.129	0.248	0.351	0.359	0.316	0.240	0.143	0.111	0.085
XYL	0	222.2b	0.091	3.12	17.0c	36.9b	63.4c	92.5c	121.8bc	148.3bc	202.4bc	224.7bc	235.3ab	242.4ab
	10	215.0b	0.087	2.97	19.5b	39.7b	65.2c	92.1c	118.9c	143.7c	195.7cd	219.4c	230.3b	237.0b
	20	218.8b	0.091	2.89	22.4a	45.2a	72.4b	99.9b	127.1b	152.3ab	205.0ab	227.5ab	237.6ab	243.6ab
	40	216.3b	0.096	3.15	22.6a	48.2a	77.5a	106.8a	133.9a	159.3a	211.4a	233.1a	242.8a	248.7a
	80	240.5a	0.069	2.50	14.5d	29.2c	51.4d	77.1d	104.3d	131.1d	192.8d	222.2bc	236.6ab	245.7ab
P value	Linear	0.563	0.836	0.528	<0.001	<0.001	<0.001	0.001	0.028	0.110	0.266	0.273	0.447	0.724
	Quadratic	0.294	0.071	0.910	0.707	0.125	0.060	0.024	0.010	0.006	0.001	0.007	0.031	0.063
SEM pooled		14.57	0.0020	0.235	1.60	1.05	6.72	2.17	2.60	12.92	13.47	13.61	13.82	14.06
P value														
	Enzyme	0.161	<0.001	0.089	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.021	0.364	0.632
	Dose:													
	Linear	0.258	0.852	0.153	<0.001	<0.001	<0.001	<0.001	0.001	0.005	0.013	0.013	0.017	0.031
	Quadratic	0.472	0.041	0.600	0.191	0.519	0.852	0.824	0.717	0.725	0.872	0.713	0.529	0.417
	Enzyme × Dose	0.012	<0.001	0.773	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.024	0.044	0.047

b, the asymptotic gas production; *c*, the rate of gas production; DM, dry matter; *L*, the initial delay before gas production begins. Means within in the same column with different superscripts differ significantly among treatments (P<0.05).

of CEL (i.e., 80 µg/g DM) increased GP compared to control. This result showed the difference between both enzymes, and importance to determine optimal doses of each enzyme. Increasing level of XYL (i.e., 80 µg/g DM) affected negatively on fermentation, which may be due to the fact that excessive levels of XYL prevented binding of enzymes to substrate receptors causing reduced microorganisms attachment to feeds (Treacher and Hunt 1996). Beauchemin *et al.* (2003) concluded that high levels of enzyme addition can be less effective than low levels, indicating the need to determine the optimal application rate of enzyme.

In vitro *fermentation kinetics:* Interaction effects were observed between enzyme type and enzyme dose for DMD (P=0.003), OMD (P=0.003), NDFD (P=0.031), ME (P=0.003), SCFA (P=0.003) and MCP production (P=0.003), which clearly showed the main factors affecting fermentation kinetic of corn stover with fibrolytic enzyme addition.

Addition of CEL and XYL linearly decreased (P=0.041) pH compared to control (Table 2). This may be due to greater enzymatic hydrolysis of feeds into readily fermentable substrates that depress pH when fermented. Elghandour *et al.* (2013) obtained decreased ruminal pH values when incubated 4 fibrous feeds, including corn stover, with different levels of exogenous fibrolytic enzyme. Linearly increased OMD (P=0.030), ME (P=0.030), SCFA (P=0.030) and MCP production (P=0.030) were obtained with CEL addition at 40 and 80 µg/g DM of corn stover. The dose of 40 µg XYL/g DM corn stover quadratically increased OMD (P=0.001), ME (P=0.024), SCFA production (P=0.001) and MCP production (P=0.001) compared to other doses (Table 2). Several studies showed that adding exogenous enzyme to ruminant diets increased feed digestion *in situ* (Togtokhbayar *et al*. 2015), *in vitro* (Salem *et al*. 2015b) or *in vivo* (Alsersy *et al*. 2015, Salem *et al*. 2015a, Valdes *et al*. 2015). Increased *in vitro* GP with enzyme is mainly due to increased OMD as GP is closely correlated with the amount of OM fermented (Elghandour *et al*. 2014). Increased OMD with enzyme addition may allow higher voluntary feed intake and overcome the problem of low intakes and slow digestion rates of low quality forages because long retention times of digesta in the rumen (Leng 1990). This can allow decreasing the physical rumen fill and stimulating MCP production (Oba and Allen 2000). The increase of OMD, ME and SCFA production of corn stover with the addition of CEL and XYL may be due to increased digestion and improved ruminal fermentation (Nsereko *et al*. 2002, Khattab *et al*. 2011), enhanced attachment and colonization between enzymes and the plant cell wall material (Morgavi *et al*. 2001). Our results also agree with Elghandour *et al*. (2013), who concluded that

Table 2. Impact of cellulase (CEL) and xylanase (XYL) at different doses on pH, digestion and fermentation of corn stover

Enzyme	Dose (µg/g DM)	pH	DMD, DM mg/g	OMD, DM mg/g	NDFD, DM mg/g	ADFD, mg/g DM	ME, MJ/kg DM	SCFA, mmol/g DM	MCP, mg/g DM
CEL	0	5.62a	525.0	492.4b	431.1	268.3	7.34b	3.88b	604.9b
	10	5.57b	501.8	518.9ab	431.8	271.2	7.75ab	4.21ab	632.7ab
	20	5.57b	504.5	520.5ab	435.4	270.7	7.77ab	4.23ab	634.4ab
	40	5.60ab	495.0	542.3a	432.5	270.6	8.10a	4.51a	657.3a
	80	5.56c	508.9	528.9a	438.5	266.5	7.90a	4.34a	643.3a
P value	Linear	0.041	0.160	0.030	0.574	0.539	0.030	0.030	0.030
	Quadratic	0.266	0.137	0.240	0.828	0.593	0.240	0.240	0.241
XYL	0	5.70a	548.2	539.6bc	445.1	275.8	8.06bc	4.47bc	654.5bc
	10	5.64ab	557.4	527.8cd	431.6	270.9	7.88cd	4.32cd	642.0cd
	20	5.64ab	563.4	544.2ab	440	273.7	8.13ab	4.53ab	659.3ab
	40	5.63ab	556.7	555.7a	434.1	271.9	8.31a	4.67a	671.4a
	80	5.62b	577.1	522.6d	421.6	264	7.80d	4.26d	636.6d
P value	Linear	0.022	0.140	0.266	0.330	0.616	0.269	0.267	0.269
	Quadratic	0.132	0.850	0.001	0.237	0.292	0.001	0.001	0.001
SEM pooled		0.016	15.56	16.18	14.52	12.75	1.10	0.077	16.50
P value									
	Enzyme	<0.001	0.306	0.001	0.835	0.306	0.001	0.001	0.001
	Dose:								
	Linear	0.002	0.629	0.013	0.934	0.968	0.013	0.013	0.013
	Quadratic	0.059	0.250	0.872	0.123	0.659	0.872	0.874	0.872
	Enzyme × Dose	0.455	0.003	0.003	0.031	0.459	0.003	0.003	0.003

ADFD, *in vitro* acid detergent fiber degradability; DM, dry matter; DMD, *in vitro* dry matter degradability; MCP, microbial crude protein production; ME, metabolizable energy; NDFD, *in vitro* neutral detergent fiber degradability; OMD, *in vitro* organic matter degradability; SCFA, short chain fatty acids. Means within in the same column with different superscripts differ significantly among treatments (P<0.05).

supplementation of enzymes to fibrous feeds increased *in vitro* OMD, ME and SCFA production.

It could be concluded that addition of exogenous fibrolytic enzymes cellulase or xylanase increased *in vitro* gas production and fermentation kinetics of corn stover. The optimum dose of both cellulase and xylanase is 40 µg/g DM; however, the difference of xylanase between treatments and control was less than that of cellulase.

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REFERENCES

Abdel-Aziz N A, Salem A Z M, El-Adawy M M, Camacho L M, Kholif A E, Elghandour M M Y and Borhami B E. 2015. Biological treatments as a mean to improve feed utilization in agriculture animals-An overview. *Journal of Integrative Agriculture* **14**: 534–43.

Alsersy H, Salem A Z M, Borhami B E, Olivares J, Gado H M, Mariezcurrena M D, Yacuot M H, Kholif A E, El-Adawy M and Hernandez S R. 2015. Effect of Mediterranean saltbush (*Atriplex halimus*) ensilaging with two developed enzyme cocktails on feed intake, nutrient digestibility and ruminal fermentation in sheep. *Animal Science Journal* **86**: 51–58.

AOAC 1997.*Official Methods of Analysis*.16th edn.Association of Official Analytical Chemists.Arlington, VA, USA.

Beauchemin K A, Collombatto D, Morgavi D P and Yang Y Z. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Science* **81**: E37–E47.

Elghandour M M Y, Salem A Z M, Gonzalez-Ronquillo M, Brquez J L, Gado H M, Odongo N E and Penuelas C G. 2013. Effects of exogenous enzymes on in vitro gas production kinetics and ruminal fermentation of four fibrous feeds. *Animal Feed Science and Technology* **179**: 46– 53.

Elghandour M M Y, Salem A Z M, Martínez Castañeda J S, Camacho L M, Kholif A E and Vázquez Chagoyán J C. 2015. Direct-fed microbes: A tool for improving the utilization of low quality roughages in ruminants. *Journal of Integrative Agriculture* **14**: 526–33.

Elghandour M M Y, Vázquez Chagoyán J C, Salem A Z M, Kholif

A E, Martínez Castañeda J S, Camacho L M and Cerrillo-Soto M A. 2014. Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on *in vitro* gas production kinetics and degradability of four fibrous feeds. *Italian Journal of Animal Science* **13**: 295–301.

France J, Dijkstra J, Dhanoa M S, López S and Bannink A. 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. *British Journal of Nutrition* **83**: 143–50.

Getachew G, Makkar H P S and Becker K. 2002. Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. *Journal of Agriculture Science* **139**: 341–52.

Giraldo L A, Ranilla M J, Tejido M L and Carro M D. 2004. Effect of enzyme application method on *in vitro* rumen fermentation of tropical forages. *Journal of Animal and Feed Sciences* **13**: 63–66.

Goering M K and Van Soest P J. 1970. *Forage fiber analysis (apparatus, reagents, procedures and some applications)*. Agriculture Handbook, No 379. Agricultural Research Service, USDA, Washington, USA.

Khattab H M, Gado H M, Kholif A E, Mansour A M and Kholif A M. 2011. The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. *International Journal of Dairy Science* **6**: 267–77.

Khattab H M, Gado H M, Salem A Z M, Camacho L M, El-Sayed M M, Kholif A M, El-Shewy A A and Kholif A E. 2013. Chemical composition and *in vitro* digestibility of *Pleurotus ostreatus* spent rice straw. *Animal Nutrition and Feed Technology* **13**: 507–16.

Kholif A E, Khattab H M, El-Shewy A A, Salem A Z M., Kholif A M, El-Sayed M M, Gado H M and Mariezcurrena M D. 2014. Nutrient digestibility, ruminal fermentation activities, serum parameters and milk production and composition of lactating goats fed diets containing rice straw treated with *Pleurotus ostreatus*. *Asian-Australasian Journal of Animal Sciences* **27**: 357–64.

Kung L, Treacher R J, Nauman G A, Smagala A M, Endres K M and Cohen M A. 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. *Journal of Dairy Science* **83**: 115–22.

Leng R A. 1990. Factors affecting the utilization of 'poor quality' forages by ruminants particularly under tropical conditions. *Nutrition Research Reviews* **3**: 277–303.

Mao H L, Wu C H, Wang J K and Liu J X. 2013. Synergistic effect of cellulase and xylanase on in vitro rumen fermentation and microbial population with rice straw as substrate. *Animal Nutrition and Feed Technology* **13**: 477–87.

Menke K H, Raab L, Salewski A, Steingass H, Fritz D and Schneider W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agriculture Science* **93**: 217–22.

Morgavi D P, Beauchemin K A, Nsereko V L, Rode L M, McAllister T A, Iwaasa A D, Wang Y and Yang W Z. 2001. Resistance of feed enzymes to proteolytic inactivation by rumen microorganisms and gastrointestinal proteases. *Journal of Animal Science* **79**: 1621–30.

Nsereko V L, Beauchemin K A, Morgavi D P, Rode L M, Furtado A F, Mcallister T A, Iwaasa A D, Yang W Z and Wang Y. 2002. Effect of a fibrolytic enzyme preparation from Trichodermalongibrachiatum on the rumen microbial population of dairy cows. *Canadian Journal of Microbiology* **48**: 14–20.

Oba M and Allen M S. 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber. 3. Digestibility and microbial efficiency. *Journal of Dairy Science* **83**: 1350–58.

Salem A Z M, Alsersy H, Camacho L M, El-Adawy M M, Elghandour M M Y, Kholif A E, Rivero N, Alonso M U and Zaragoza A. 2015a. Feed intake, nutrient digestibility, nitrogen utilization and ruminal fermentation activities of sheep fed *Atriplex halimus* ensiled with three developed enzyme cocktails. *Czech Journal of Animal Science* **60**: 185–94.

Salem A Z M, Ammar H, Kholif A E, Elghandour M M Y and Ortiz L B. 2015b. Effect of glucoamylase enzyme extract on *in vitro* gas production and degradability of two diets with 25% of corn or sorghum grains. *Indian Journal of Animal Sciences* **85**: 183–88.

SAS Institute. 2002. *SAS User's Guide: Statistics.Ver 9.0*. SAS Institute, Cary, N.C. USA. 956 pp.

Theodorou M K, Williams B A, Dhanoa M S, McAllan A B and France J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology* **48**: 185–97.

Togtokhbayar N, Cerrillo M A, Rodríguez G B, Elghandour M M Y, Salem A Z M, Urankhaich C, Jigjidpurev S, Odongo N E, Kholif A E. 2015. Effect of exogenous xylanase on rumen *in vitro* gas production and degradability of wheat straw. *Animal Science Journal* **86**: 765–71. doi: 10.1111/asj.12364.

Treacher R J and Hunt C W. 1996. Recent developments in feed enzymes for ruminant rations. *Proceedings of Pacific Northwest Animal Nutrition Conference*, Seattle, WA, USA, pp. 37–54.

Valdes K I, Salem A Z M, Lopez S, Alonso M U, Rivero N, Elghandour M M Y, Domínguez I A, Ronquillo M G and Kholif A E. 2015. Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. *Journal of Agriculture Science* **153**: 732–42.

Van Soest P J, Robertson J B and Lewis B A. 1991.Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.

7.3 Producción de gas, metano, dióxido de carbono *in vitro* y cinética de fermentación de una ración mixta concentrada suplementada con xilanasa y *S. cerevisiae* incubada con líquido ruminal de cabras, borregos y novillos

In vitro gas, methane and carbon dioxide productions, as well as ruminal microflora and fermentation kinetics of a high concentrates total mixed ration supplemented with xylanase and _S. cerevisiae_ and incubated with rumen liquor from goats, sheep and steers

L.H. Vallejo [a], A.E. Kholif [b], M.M.Y. Elghandour [a], A.Z.M. Salem [a,*]

[a] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, México

[b] Dairy Science Department, National Research Centre, 33 Bohouth St. Dokki, Giza, Egypt

*Corresponding author: asalem70@yahoo.com (A.Z.M. Salem)

Short title: *Yeast and xylanase affects ruminal fermentation*

Highlights

- Yeast and xylanase did not affect gas production
- Yeast and xylanase decreased methane production
- Yeast and xylanase increased ruminal bacteria without affecting ruminal protozoa
- Fermentation patterns differed between goat, sheep, and steers

Abstract

The aim of this study was to study the effect of *S. cerevisiae* yeast, xylanase, and their mixture on ruminal fermentation of a high concentrate ration (basal ration) using inoculum from different sources. Rumen liquor from two cannulated Holstein steers, two cannulated Creole goat and two cannulated Rambouillet sheep. The basal ration was supplemented (per g dry matter (DM)) with xylanase at 2 mL, *S. cerevisiae* at 4 mg, or their mixture at 2 mL xylanase + 4 mg *S. cerevisiae*. No inoculum source × additive type interactions were observed ($P>0.05$) in this study. Most of determined parameters were affected ($P<0.05$) by the inoculum source and additive type. Additives increased ($P=0.045$) the asymptotic GP with sheep, with no effect on goat and steer inoculums. Besides, additives increased ($P<0.05$) the rate of GP with goat and sheep inoculums. *S. cerevisiae* or/and xylanase decreased ($P<0.05$) the proportional CH_4 with all inoculum sources, while increased ($P<0.05$) CO_2 production. Increased ($P<0.05$) bacterial numbers were observed with the inclusion of the additives. Short chain fatty acids and metabolizable energy concentrations were increased ($P<0.05$) when the additives were supplemented to goat and sheep inoculums. Moreover, additives increased ($P<0.05$) DM degradability with sheep and steers ($P=0.037$), and increased ($P=0.048$) organic matter degradability with goat inoculum. It is concluded that *S. cerevisiae*, xylanase and their

mixture did not affect total GP while made a desirable qualifying changes in the produced biogases. Additives decreased CH_4 production, thus, can be used as a sustainable strategy to reduce greenhouse gases from livestock.

Keywords: Feed additives, greenhouse gases, in vitro fermentation, xylanase exogenous enzyme, yeast.

1. Introduction

Recently, earth became more wormer due to the large production of greenhouse gases. Livestock production is responsible for about 18% of methane (CH_4) emission, and 9% of carbon dioxide (CO_2) production (FAO, 2006). Methane and CO_2 are the result of ruminal fermentation of feeds, causing losing of 2 to 12% of gross dietary energy (Hristov et al., 2015). Attempts including the inclusion of *S. cerevisiae* (Elghandour et al., 2017), organic acids salt (Elghandour et al., 2016), exogenous enzymes (Kholif et al., 2017), and essential oils (Hernandez et al., 2017), have been used to address the sustainable ruminal CH_4 and CO_2 emissions from ruminant feeds. Based on the energy balances reported by Bruinenberg et al. (2002) and Nkrumah et al. (2006), reducing CH_4 emission could potentially increase body weight gain of growing cattle by 75 g/d and milk production in dairy cows by approximately 1 L/d.

Because the European Union banned the inclusion of antibiotics and ionophores as feed additive in animal feeding, exploring alternative natural feed additives to modify ruminal fermentation and enhance feed utilization (Salem et al., 2014), and reducing the emission of GHG (Elghandour et al., 2017; Kholif et al., 2017). Studies have shown that the inclusion of exogenous enzymes in ruminants feeding improved productive performance of animal due to improving nutrient digestibility, and ruminal fermentation (Valdes et al., 2015). Researcher proposed some modes of actions of improved feed utilization including solubilization of dietary fiber, supplementing ruminal microorganisms with readily fermentable substrate, enhancing of microbial enzyme activity in the rumen (McAllister et al. 2001), and enhancing the attachment and colonization of ruminal microorganisms to the plant cell wall (Chung et al. 2012). However, Lewis et al. (1999) observed weak effects of feeding exogenous enzymes to enhance forage quality and utilization by ruminants. The inconsistency is a result of different sources of the enzyme (Khattab et al., 2011), different doses and activities of the

enzyme (Morsy et al., 2016), different physical properties of diets (Elghandour et al., 2015), and enzyme application method (Elghandour et al., 2016) as well as level of animal productivity (Beauchemin et al., 2003).

The inclusion of *S. cerevisiae* yeast offers a great potential for manipulating ruminal fermentation in vitro (Elghandour et al., 2014) and in vivo (Ahmed et al., 2015). *S. cerevisiae* inclusion in diet of animals enhanced nutritional value of poor quality forages (Ahmed et al., 2015), nutrient digestibility (Hassan et al., 2016), and animal carcass characteristics (Velázquez-Garduño et al., 2015). Rodriguez et al. (2015) observed an increased in vitro rumen degradability and gas production (GP) of feed with *S. cerevisiae* addition. Few studies investigated the using of natural feed additives such as *S. cerevisiae* and xylanase on GHG productions; therefore, the effect of *S. cerevisiae*, xylanase and their mixture on biogases production, ruminal microbial population, and rumen fermentation of a high concentrate ration using rumen inoculum from goats, sheep, and steers.

2. Materials and methods

2.1. Substrate and treatments

A total mixed ration (TMR) was prepared as a substrate to contain (per kg DM) 520 g ground sorghum grain, 300 g corn stover, 60 g soybean meal, 80 g molasses, and 40 g urea. The chemical composition of the TMR was as (per kg DM): 880 g DM (wet weight basis), 963 g organic matter (OM), 183 g crude protein (CP), 304 g neutral detergent fiber (NDF), and 261 g acid detergent fiber (ADF). The TMR without additive was considered as a control treatment. The basal TMR was supplemented (per g DM substrate) with xylanase at 2 mL, *S. cerevisiae* at 4 mg, or their mixture at 2 mL xylanase + 4 mg *S. cerevisiae*.

99 *2.2. In vitro fermentation and biodegradation*

100 Rumen inoculum was collected from two cannulated Holstein steers (450 ± 20 kg body
101 weight (BW)), two cannulated Rambouillet sheep (60 ± 2 kg BW), and two cannulated
102 Creole goat (50 ± 2 kg BW), housed in individual pens and fed *ad libitum* on a diet consisting
103 of oat hay and concentrate (PURINA®, Toluca, Mexico) at 60:40 ratio, with free access to
104 water. Animals were fed twice daily at 08:00 and 16:00 h, and managed under the conditions
105 stipulated in the Official Mexican Standard of technical specifications for the production,
106 care and use of laboratory animals (NOM-062-ZOO-1999). Rumen contents were placed in a
107 plastic thermo preheated at 39°C, and transport to the laboratory where flushed with CO_2,
108 mixed and strained through four layers of cheesecloth into a flask with oxygen-free
109 headspace, and maintained at a constant temperature of 39°C and continuous flow of CO_2.

110 Before the incubation process, incubation medium containing buffer, macromineral,
111 micromineral and resarzurin solutions, and distilled water were prepared according to
112 Goering and Van Soest (1970), mixed in a volumetric flask, using a platen and magnetic
113 stirrer at 39°C to maintain the temperature and homogenize the solution. After, the ruminal
114 inoculum and the reducing solution were added at 1:4 vol/vol, respectively.

115 Samples (0.5 g) of the substrate were weighed into 120 mL serum bottles with
116 appropriate addition of the additives (i.e., xylanase or/and *S. cerevisiae*). Consequently, 50
117 mL of previously prepared rumen liquor and the buffer were added. Bottles were maintained
118 at constant CO_2 flow for 30 sec, and then capped with neoprene plugs and then sealed with
119 aluminum rings. The vials were placed in an incubator (Riossa®, F-51 D, Mexico State,
120 Mexico) at 39°C for 48 h. Moreover, three bottles as blanks (rumen fluid only) were
121 incubated for 48 h. Three incubation runs were performed in three weeks.

Gas production readings were performed at 2, 4, 6, 8, 10, 12, 24 and 48 h of incubation using water displacement apparatus according to Fedorak and Hrudey (1983). The apparatus was designed with a universal support, with a conical funnel, a 100 mL burette and two latex hoses of 0.5 and 1 m in length and 3/8 inch diameter. The vials were punctured with a 16 gauge needle placed at the end of the hose. Gas production (mL) was measured by the displacement of water in the burette.

After 48 h of incubation, 5 ml of gas were taken and stored in the vials with saturated saline solution prepared with 400 g of NaCl in 1 L of distilled water, pH adjusted at 2 and 5 mL of 20% methyl orange was added as indicator, for CH_4 and CO_2 concentrations determination. The saturated saline solution was previously prepared and stored in 60 mL serological vials, leaving no space; and neoprene plugs were placed and sealed with aluminum rings, and stored away from light. For the determination of CH_4 and CO_2, from the vials with saturated saline a sample of 10 μL of the gas phase was taken and injected into a PerkinElmer, Claurus 500 gas chromatograph (Mexico City, Mexico) with a flame ionization detection, and helium was the carrier gas. A thermal conductivity detector was used, the oven, column and TCD temperatures were 80°C, 170°C and 130°C respectively. Retention times were 0.73 min and 1.05 min for CH_4 and CO_2 respectively.

At the end of incubation at 48 h, the fermentation process was stopped by swirling the bottles in ice for 5 minutes, then the bottles were uncapped and the pH was measured using a pH meter (Thermo Scientific, Orion Star™ A121, Beverly, MA, USA). The contents of the bottles were filtered in Ankom® Technologies F57 bags (at constant weight), with the aid of a filtration system connected to a vacuum pump. The bottles were rinsed with a hot water 3 times to ensure recovery of all the residue of the fermentation. The bags were placed in a forced air oven at 55 °C for 48 h. Dry matter degradability (DMD) was calculated by considering the initial weight of the substrate and the weight of the residue.

147 After pH measure and filtration, 4 mL of the medium were obtained with a syringe and
148 mixed with 1 mL of 25% metaphosphoric acid, shaken slightly and placed under freezing
149 until analysis of NH_3-N concentration. Other 4 mL of the medium were mixed with 1 mL
150 10% formaldehyde and shaken slightly then placed in refrigeration until analysis of bacterial
151 and protozoal counting.

152

153 *2.3. Total bacteria and protozoa counting*

154 The concentration of total bacteria was determined at 48 h of incubation using a count
155 chamber bacterium Petroff-Hausser (Hausser Scientific®, 3900, Horsham, PA) and a phase
156 contrast microscope (Olympus®, BX51, Mexico City, Mexico) at a magnification of 100×.
157 Exactly, 0.5 mL of the 10% formaldehyde fixed medium sample was taken and diluted in 4.5
158 mL of distilled water. The concentration of bacteria per mL was determined as the average of
159 bacteria observed in each grid, multiplied by the dilution factor and the chamber factor
160 (2×10^7), according to the following formula: $\text{Bacterial number/mL} = \mu\times FD1\times FD2\times 2^7$

161 Where: μ is the average of bacteria in each grid per treatment, FD1 is the first dilution factor
162 (1.25), and FD2 is the second dilution factor (10)

163 For the protozoal number determination, 1 mL of the 10% formaldehyde fixed sample
164 was obtained and diluted in 1 mL of distilled water, then 0.5 mL of the mixture was taken
165 with a Pasteur pipette (BRAND, 7712, Wertheim, Germany), which were deposited in a
166 Neubauer chamber (BRAND, 7178-10, Wertheim, Germany), subsequently observed on a
167 contrast microscope (Carl Zeiss®, Axiostar, Mexico City, Mexico) at 400× magnifications.
168 The count of protozoa was made in eight quadrants (4 of each grid), taking as viable
169 protozoans those that maintained their morphological integrity. The concentration of protozoa
170 per mL of culture medium was estimated as the average of protozoa observed in each grid,

multiplied by the dilution factor and the chamber factor (1×10^4), according to the following formula: $\text{Protozoal number} = \mu \times \text{FD1} \times \text{FD2} \times 10^4$

Where: = μ is the average of protozoa in each grid per treatment, FD1 is the first dilution factor (5), and FD2 is the second dilution factor (3).

2.4. Chemical analyses

Samples of the TMR were analyzed for DM (#934.01), ash (#942.05), N (#954.01) and ether extract (EE; #920.39) according to AOAC (1997), while TMR's contents for NDF content (Van Soest et al., 1991), ADF and lignin (AOAC, 1997; #973.18) analyses were carried out using an ANKOM^{200} Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA) with the use of an alpha amylase and sodium sulfite.

The concentration of ruminal NH_3-N was determined according to Broderick and Kang (1980) method. Sample of the incubation medium were centrifuged at 3000×g for 10 min, and 20 µl of the supernatant was mixed with 1 mL of phenol and 1 mL of hypochlorite, then mixture and incubated at 39 °C for 30 min, after were diluted with 5 mL of distilled water. Samples were read on a visible ultraviolet light spectrophotometer (Varian, model Cary 1E, California, USA) at 630 nm. The resulting mg/dL concentration was divided by 0.8 which is the 25% metaphosphoric acid dilution factor.

2.4. Calculations and statistical analyses

For estimation of GP kinetic, recorded gas volumes (mL/g DM) were fitted using the NLIN procedure of SAS (2002) according to France et al. (2000) model as:

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

where y is the volume of GP at time t (h); b is the asymptotic GP (mL/g DM); c is the fractional rate of fermentation (/h), and L (h) is the discrete lag time prior to any gas is released.

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

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

(3) 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 (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:

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

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

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

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

Microbial biomass production (MCP) was calculated (Blümmel et al., 1997) as:

(6) MCP (mg/g DM) = Milligrams DMD − (Milliliter gas × 2.2 mg/mL)

where the 2.2 mg/mL is a stoichiometric factor that expresses mg of C, H and O required for the SCFA gas associated with production of 1 mL of gas (Blümmel et al., 1997).

216

217 *2.5. Statistical analyses*

218 Data of each of the three runs within the same sample of each of the three individual
219 samples of rations were averaged prior to statistical analysis, then mean values of each
220 individual sample were used as the experimental unit. The experimental design was a
221 factorial design with 3 replicates in a randomized complete block design. Data were analyzed
222 using the GLM procedure (SAS, 2002) with the model: $Y_{ijk} = \mu + R_i + A_j + (R \times A)_{ij} + \varepsilon_{ijk}$
223 where: Y_{ijk} is the observation, μ is the population mean, R_i is the inoculum source effect, A_j is
224 the type of feed additives, $(R \times A)_{ij}$ is the interaction between feed additive type and
225 inoculum source, and ε_{ijk} is the residual error. Tukey test was used to compare means.

226

227 **3. Results**

228 *3.1. Gases production*

229 No inoculum source × additive type interactions were observed for all parameters of
230 GP, CH_4 and CO_2 productions (Table 1). Gas production, CH_4, and CO_2 productions were
231 differed ($P<0.05$) between goats, sheep, and steers. The type of feed additives affected
232 ($P<0.05$) GP and CH_4 production.

233 Fig 1 shows GP at different incubation hours and affected with different inoculum
234 sources and the different feed additives. The inclusion of *S. cerevisiae*, enzyme or their
235 mixture did not affect ($P>0.05$) the asymptotic GP with goat and steer rumen inoculum;
236 however, increased ($P=0.045$) the asymptotic GP with sheep (Table 1). Increased rate of GP
237 were obtained with the addition of *S. cerevisiae*, enzyme and their mixture to the ration with
238 goat ($P=0.48$) and sheep ($P=0.046$) inoculums. Without affecting the lag time of GP with goat

239 and sheep inoculums (P>0.05), the used additives decreased (P=0.029) the lag time of GP of
240 steers.

241 The used additives did not affect CH_4 production (mL/g DM); however, the same
242 additives decreased the proportional CH_4 from goat (P=0.046), sheep (P=0.04), and steers
243 (P=0.041), as well as CH_4 production as mL/g DM from goats (P=0.041) and sheep
244 (P=0.024) (Table 1). At the same time, the inclusion of *S. cerevisiae* or/and xylanase
245 increased (P<0.05) CO_2 production as mL/g incubated and degraded DM, and as a percent of
246 total GP.

247

248 *3.2. Microbial population*

249 An interaction between inoculum source and additive type was observed (P<0.001) for
250 total protozoal number; however, the interaction was absent (P>0.05) for total bacterial
251 number (Table 2). Additive type did not affect (P>0.05) both of total bacterial and protozoal
252 numbers; however, protozoal number differed (P=0.002) between inoculum sources. For all
253 inoculum sources, total bacterial numbers were increased (P<0.05), while total protozoal
254 numbers were not affected (P>0.05) with the addition of *S. cerevisiae*, xylanase and their
255 mixture.

256

257 *3.3. Fermentation kinetics*

258 No interactions were observed (P>0.05) between rumen liquor donor and additive type
259 for all determined parameters of fermentation kinetics (Table 2). Most of determined
260 fermentation parameters differed (P<0.05) between goat, sheep and steer inoculums, and also
261 between feed additive types. No effect was observed (P>0.05) with the addition of *S.*

cerevisiae or/and xylanase on ruminal pH, NH3-N concentration, PF_{24}, and GY_{24}. Increased SCFA concentrations were observed with the inclusion of the additives with goat (P=0.041) and sheep (P=0.45) inoculums. The used additives increased DMD with sheep (P=0.009) and steers (P=0.037), while increased (P=0.048) OMD with goat inoculum. *S. cerevisiae* or/and xylanase increased ME concentration with goats (P=0.019) and sheep (P=0.046), and also increased MCP (P=0.042) with goat inoculum.

4. Discussion

4.1. Gas production

The absent of inoculum type and type of feed additives reveals that the effect of each additive will not be inoculum dependent. Besides, parameters of GP differed between goat, sheep, and steers revealing that using rumen fluid from different ruminant species for the in vitro evaluation of feed is strongly recommended. Aderinboye et al. (2016) observed different fermentation parameters among cows, sheep and goats inoculums due to differed bacterial and protozoal populations and microbial activity between goats, sheep and steers (Aderinboye et al., 2016). Based on the fact that ruminal microbial population depends mainly on the type of diet fed, and since all of goats, sheep and steers were maintained on the same diet, microbial species were not expected to vary (Mould et al., 2005). At the same time, Mould et al. (2005) reported some factors which might cause some variations in inoculum including host animal effects, and preparation of sample and inoculation. Moreover, Ammar et al. (2004) reported other factor which may cause some variations between inoculums from different animal species, including chewing/eating behavior, gut physiology, compartment dimensions and retention time will influence gut microflora.

285 Gas production differed between the tested feed additives. This was expected because each of
286 them has a different mode of action to affect ruminal fermentation (Hernandez et al., 2017).
287 However, many reports observed that inclusion of *S. cerevisiae* (Elghandour et al., 2014) and
288 enzymes (Vallejo et al., 2016) was paralleled with increased GP, *S. cerevisiae* and xylanase
289 had a weak effect on GP from goat and steer, while increased it from sheep. In same line of
290 the present results, Hernandez et al. (2017) showed negligible effects with exogenous
291 xylanase and *S. cerevisiae* on GP kinetics. The differed response between rumen liquor
292 donors can be explained based on differed ruminal microflora between species, as previously
293 explained.

294 The tested additives increased rate of GP and decreased the lag time of GP revealing
295 better nutrients utilization. Recent studies showed that exogenous enzymes inclusion in diets
296 of ruminants improved feed utilization, digestion of DM, and animal performance by
297 improving DM degradation (Morsy et al., 2016). Rodriguez et al. (2015) observed that *S.*
298 *cerevisiae* addition decreased rate of GP. The inconstancy may be due to the composition of
299 the substrates (Elghandour et al., 2014). The decreased lag time of GP may be due to
300 increased degradation of feed nutrients especially fibers (Kholif et al., 2016; Elghandour et
301 al., 2017). Exogenous enzymes have the ability to stimulate the initial phases of microbial
302 colonization in the rumen and facilitate the bacterial attachment to feed particles (Giraldo et
303 al., 2007). *S. cerevisiae* was reported to effectively consume O_2 molecules from the rumen
304 making the ruminal environment more commensurate for optimum activity of various
305 microorganisms (Newbold et al., 1996). In addition, Callaway and Martin (1997) reported
306 that *S. cerevisiae* contains small peptides and many important nutrients required for the
307 growth and activity of ruminal microorganisms especially ruminal cellulolytic bacteria to
308 initiate the fermentation process (Paya et al. 2007). Williams et al. (1991) reported a
309 stimulation effect of *S. cerevisiae* on cellulose degradation, which was associated with a

310 decreased lag time and increased initial rates of digestion, without affecting the extent of
311 ruminal digestion. Decreasing the lag time of GP with xylanase and *S. cerevisiae* reveals the
312 ability of these additives to overcoming the problem of low intakes and slow digestion of low
313 quality forages (Salem et al., 2015).

314

315 *4.2. Greenhouse gases*

316 The production of CH_4, and CO_2 differed between goats, sheep, and steers, which may
317 be due differed ruminal microflora population. Hook et al. (2010) observed differed CH_4
318 production from different ruminant species. Boeckaert et al. (2007) reported that ruminal
319 protozoal population is animal-to-animal varies, despite the feeding of the same diets. The
320 varied CH_4 production between ruminal species indicates the fact that one species could not
321 be used to predict CH_4 production (Bueno et al., 2005). As previously shown, enteric CH_4
322 emission contributes to a loss of net feed energy (Hristov et al., 2015). Therefore, intensive
323 research efforts are recently directed towards ruminant animals CH_4 mitigation (Elghandour
324 et al., 2016b), and any strategy to mitigate CH_4 production from livestock sector is always
325 desirable. During ruminal fermentation process within the rumen, gases consisting of mainly
326 CH_4, CO_2 and H_2 are produced. Therefore, the unaffected GP and decreased the proportional
327 CH_4 production reveals that the additives were effective to reduce CH_4 production, and may
328 serve as efficient methods to reduce CH_4 emission from ruminant production. Polyorach et al.
329 (2014) observed that *S. cerevisiae* supplementation decrease in *in vitro* CH_4 production,
330 which supports our findings. Moreover, Salem et al. (2015) and Kholif et al. (2016) observed
331 that the addition of enzymes decreased CH_4 production in equine diets. In ruminant nutrition,
332 xylanase was not expected to reduce CH_4 production because is supposed that xylanase will
333 increase the availability of hemicellulose and CH_4 production (Elghandour et al., 2016b); the
334 reason is not clear. The declined CH_4 production can be explained based on the ability of
335 xylanase to stimulate the reductive acetogens in the rumen that alters H_2 metabolism and its
336 utilization by methanogens in a manner that reduces CH_4 formation and emissions (Stewart et
337 al., 1997).

338 *S. cerevisiae* reduced CH_4 production because the ability of *S. cerevisiae* to stimulate
339 the acetogens through competing and co-metabolizing H_2 with methanogens (Hristov et al.,
340 2013). Moreover, the inclusion of *S. cerevisiae* in the diets of ruminants proved to enhance
341 nutrient digestibility (Hassan et al., 2016) and altering SCFA production in the rumen by
342 elevating populations of cellulolytic and amylolytic bacteria in the rumen (Kumar et al.,
343 1997). The full mode of action of reducing CH_4 production is not clear, because some studies
344 reported increases in CH_4 production with *S. cerevisiae* supplementation (Elghandour et al.,
345 2017). Newbold and Rode (2006) reported a decreased CH_4 production with feeding live *S.*
346 *cerevisiae* products.

347 From the previous explanations, it is clear that both of *S. cerevisiae* and xylanase act on
348 the metabolization of H_2 in the rumen because a copious quantity of H_2 is produced and
349 together with CO_2 from the ruminal degradation of organic matter and be used to synthesize
350 CH_4 by methanogenic Archaea (Hernandez et al., 2017).

351

352 *4.3. Microbial population*

353 Additives did not affect total protozoal numbers. Corona et al. (1999) and Chung et al.
354 (2012) reported unaffected protozoa population with *S. cerevisiae* and fibrolytic enzyme
355 supplementation administration in rams and cows. *S. cerevisiae* and xylanase increased total
356 bacterial numbers. Newbold et al. (1996) observed that *S. cerevisiae* supplementation caused
357 increases in the number of total anaerobic and cellulolytic bacteria. The increase bacterial
358 number with *S. cerevisiae* is a result of providing the incubation medium with important
359 nutrients, nutritional cofactors, and vitamins such as biotin and thiamine, which are required
360 for enhancing microbial growth and activity (Callaway and Martin, 1997). Besides, *S.*
361 *cerevisiae* provides conducive anaerobic conditions to microbial growth (Mosoni et al. 2007),

362 making the ruminal environment more suitable for microbial growth. On the other hand,
363 exogenous enzyme, like xylanase, can stimulate ruminal fibrolytic and non-fibrolytic bacteria
364 through releasing of carbohydrates that are readily utilized by the bacteria (Nsereko et al.
365 2002). Increased bacterial numbers with the inclusion of cellulase and xylanase in the
366 incubation medium in vitro was observed by Mao et al. (2013).

367

368 *4.4. Fermentation kinetics*

369 Fermentation parameters differed between goat, sheep and steer inoculums, and we
370 already explained the varied response between goats, sheep and steers. Greater SCFA
371 concentrations were observed with the inclusion of the additives to goat and sheep inoculums.
372 Mao et al. (2013) reported that the inclusion of *S. cerevisiae* in the diet of ruminant increased
373 total SCFA and propionic acid production. Greater SCFA production and ME concentrations
374 are associated with enhanced activities of ruminal microflora. As previously shown, the
375 increased ruminal bacterial number with the used additives is a reason for enhanced ruminal
376 fermentation. At the same time, the improved DMD with sheep and steers, and increased
377 OMD with goat inoculum might be a result of enhanced fungal colonization on plant cell
378 walls resulting in enhanced DM and fiber digestion (Patra, 2012). The greater degradability is
379 related to ability of xylanase to enhance the attachment and colonization of ruminal microbes
380 to plant cell wall particles (Nsereko et al. 2002). Moreover, the inclusion of exogenous
381 enzyme enhances the synergism interaction between endogenous ruminal enzymes and the
382 exogenous enzyme resulting in enhanced nutrient degradability. The enhanced degradability
383 with the inclusion of *S. cerevisiae* may be a result of enhanced ruminal environment with *S.*
384 *cerevisiae* supplementation (Newbold et al., 1996; Callaway and Martin, 1997). Hernandez et

385 al. (2017) reported that the inclusion of *S. cerevisiae* and xylanase did not affect on DMD,
386 while decreased fermentation.

387

388 **5. Conclusion**

389 *S. cerevisiae* and fibrolytic enzyme xylanase did not affect gas production of the tested
390 ration; however, made a qualitative changes in produced gases. *S. cerevisiae* and xylanase
391 decreased CH_4 production, which is very important from an environmental view, and
392 therefore, these feed additives can be used as a sustainable strategy to reduce greenhouse
393 gases from livestock. Further research is needed to test more doses of the additives in both in
394 vitro and in vivo scales to validate or negate the present results.

395

396 **Conflict of interest**

397 All authors declare that there are no present or potential conflicts of interest among the
398 authors and other people or organizations that could inappropriately bias their work.

399

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405

406 **References**

407 Aderinboye, R.Y., Akinlolu, A.O., Adeleke, M.A., Najeem, G.O., Ojo, V.O.A., Isah, O.A.
408 and Babayemi, O.J., 2016. In vitro gas production and dry matter degradation of four
409 browse leaves using cattle, sheep and goat inocula. Slovak J. Anim. Sci. 49, 32-43.

410 Ahmed, M.H., Elghandour, M.M.Y., Salem, A.Z.M., Zeweil, H.S., Kholif, A.E., Klieve,
411 A.V., Abdelrassol, A.M.A., 2015. Influence of *Trichoderma reesei* or *Saccharomyces*
412 *cerevisiae* on performance, ruminal fermentation, carcass characteristics and blood
413 biochemistry of lambs fed *Atriplex nummularia* and *Acacia saligna* mixture. Livest.
414 Sci. 180, 90-97.

415 Ammar, H., Ranilla, M.J., Tejido, M.L., Ovejero, F.J., Gonzalez, J.S. Lopez, S. 2004. Effect
416 of inoculum source (sheep or goat rumen fluid) on in vitro digestibility and gas
417 production kinetics of the foliage of some Spanish browse plants. Options
418 Mediterraneennes. Serie A, Seminaires Mediterraneenes. Centre International de
419 Hautes Etudes Agronomiques Mediterraneens, Montpellier, France. 59, pp. 121–126.

420 AOAC, 1997. Association of Official Analytical Chemists. Official Methods of Analysis,
421 16th ed. AOAC, Arlington, VA, USA.

422 Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z., 2003. Use of exogenous
423 fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81: E37-E47.

424 Blümmel, M., Steingss, H., Becker, K., 1997. The relationship between in vitro gas
425 production, in vitro microbial biomass yield and ^{15}N incorporation and its implications
426 for the prediction of voluntary feed intake of roughages. Br. J. Nutr. 77, 911–921.

427 Boeckaert, C., Vlaeminck, B., Mestdagh, J. and Fievez, V., 2007. In vitro examination of
428 DHA-edible micro algae: 1. Effect on rumen lipolysis and biohydrogenation of linoleic
429 and linolenic acids. Anim. Feed Sci. Technol., 136(1), pp.63-79.

430 Bruinenberg, M. H., Y. van der Honing, R. E. Agnew, T. Yan, A. M. van Vuuren, and H.
431 Valk. 2002. Energy metabolism of dairy cows fed on grass. Livest. Prod. Sci. 75:117-
432 128.

433 Bueno, I., Abdalla, A., Cabral Filho, S.L.S., Vitti, D., Owen, E., Mauricio, R., Givens, I.,
434 Sutton, J. And Mould, F., 1999. Comparison of inocula from sheep and cattle for the in
435 vitro gas production under tropical conditions. In: Annual Meeting of The British
436 Society of Animal Science (Vol. 13, pp. 151-156).

437 Callaway, E.S., Martin, S.A., 1997. Effects of a *Saccharomyces cerevisiae* culture on ruminal
438 bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

439 Chung Y.H., Zhou M., Holtshausen L., Alexander T.W., McAllister T.A., Guan L.L., Oba
440 M., Beauchemin K.A. (2012): A fibrolytic enzyme additive for lactating Holstein cow
441 diets: ruminal fermentation, rumen microbial populations, and enteric methane
442 emissions. J. Dairy Sci. 95, 1419–1427.

443 Corona, L., Mendoza, G.D., Castrejón, F.A., Crosby, M.M. and Cobos, M.A., 1999.
444 Evaluation of two yeast cultures (*Saccharomyces cerevisiae*) on ruminal fermentation
445 and digestion in sheep fed a corn stover diet. Small Rumin. Res. 31(3), 209-214.

446 Elghandour M.M.Y., Kholif, A.E., Hernández, J., Mariezcurrena, M.D., López, S., Camacho,
447 L.M., Márquez, O., Salem, A.Z.M., 2016b. Influence of the addition of exogenous
448 xylanase with or without pre-incubation on the in vitro ruminal fermentation of three
449 fibrous feeds. Czech J. Anim. Sci. 61 (6), 262–272.

450 Elghandour, M.M., Chagoyán, J.C.V., Salem, A.Z., Kholif, A.E., Castañeda, J.S.M.,
451 Camacho, L.M. and Cerrillo-Soto, M.A., 2014. Effects of *Saccharomyces cerevisiae* at
452 direct addition or pre-incubation on in vitro gas production kinetics and degradability of
453 four fibrous feeds. Ital. J. Anim. Sci. 13(2), 295-301.

454 Elghandour, M.M.M.Y., Kholif, A.E., Marquez-Molina, O., Vazquez-Armijo, J.F., Puniya,
455 A.K. and Salem, A.Z.M., 2015. Influence of individual or mixed cellulase and xylanase
456 mixture on in vitro rumen gas production kinetics of total mixed rations with different
457 maize silage and concentrate ratios. Turk. J. Vet. Anim. Sci. 39(4), 435-442.

458 Elghandour, M.M.Y., Kholif, A.E., Salem, A.Z.M., de Oca, R.M., Barbabosa, A.,
459 Mariezcurrena, M. and Olafadehan, O.A., 2016a. Addressing sustainable ruminal
460 methane and carbon dioxide emissions of soybean hulls by organic acid salts. J. Clean.
461 Prod. 135, 194–200.

462 Elghandour, M.M.Y., Vázquez, J.C., Salem, A.Z.M., Kholif, A.E., Cipriano, M.M.,
463 Camacho, L.M., Márquez, O., 2017. In vitro gas and methane production of two mixed
464 rations influenced by three different cultures of *Saccharomyces cerevisiae*. J. Appl.
465 Anim. Res., 45, 389-395,

466 FAO (Food and Agriculture Organization of the United Nations), 2006. Livestock a Major
467 Threat to the Environment: Remedies Urgently Needed. [accessed 26 December 2016].
468 Available:http://www.fao.org/newsroom/en/news/2006/1000448/index.html.

469 Fedorak, P.M., Hrudey, S.E., 1983. A simple apparatus for measuring gas-production by
470 methanogenic cultures in serum bottles. Environ. Technol. Lett. 4, 425–432.

471 Getachew, G., Makkar, H.P.S., Becker, K., 2002. Tropical browses: contents of phenolic
472 compounds, in vitro gas production and stoichiometric relationship between short chain
473 fatty acid and in vitro gas production. J. Agr. Sci., Cambridge., 139, 341–352.

474 Giraldo, L.A., Tejido, M.L., Ranilla, M.J., Carro, M.D., 2007. Effects of exogenous cellulase
475 supplementation on microbial growth and ruminal fermentation of a high-forage diet in
476 Rusitec fermenters. J. Anim. Sci. 85, 1962–1970.

477 Goering, M.K., Van Soest, P.J., 1970. Forage Fiber Analysis (Apparatus, Reagents,
478 Procedures and Some Applications). Agriculture Handbook, No 379. Agricultural
479 Research Service, USDA, Washington, DC.

480 Hassan, A.A., Salem, A.Z.M., Kholif, A.E., Samir, M., Yacout, M.H., Hafsa, S.A., Mendoza,
481 G.D., Elghandour, M.M.Y., Ayala, M., Lopez, S., 2016. Performance of crossbred
482 dairy Friesian calves fed two levels of *Saccharomyces cerevisiae*: intake, digestion,
483 ruminal fermentation, blood parameters and faecal pathogenic bacteria. J. Agr. Sci. 154,
484 1488-1498.

485 Hernandez, A., Kholif, A.E., Lugo-Coyote, R., Elghandour, M.M.Y., Cipriano, M.,
486 Rodríguez, G.B., Odongo, N.E., Salem, A.Z.M., 2017. The effect of garlic oil, xylanase
487 enzyme and yeast on biomethane and carbon dioxide production from 60-d old Holstein
488 dairy calves fed a high concentrate diet. J. Clean. Prod. 142, 2384–2392.

489 Hook, S.E., Wright, A.D.G., McBride, B.W., 2010. Methanogens: methane producers of the
490 rumen and mitigation strategies. Archaea, 2010. Article ID 945785, 11 pages;
491 doi:10.1155/2010/945785.

492 Hristov, A. N., Oh, J., Giallongo, F., Frederick, T. W., Harper, M. T., Weeks, H. L., Branco,
493 A.F., Moate, P.J., Deighton, M. H., Williams, S.R.O., Kindermann, M., Duval, S.,
494 2015. An inhibitor persistently decreased enteric methane emission from dairy cows
495 with no negative effect on milk production. Proc. Nat. Acad. Sci. USA. 112(34),
496 10663–10668.

497 Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.,
498 Adesogan, A.T., Yang, W., Lee, C., Gerber, P.J., Henderson, B., Tricarico, J.M., 2013.
499 Special topics: mitigation of methane and nitrous oxide emissions from animal

operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045–5069.

Khattab, H.M., Gado, H.M., Kholif, A.E., Mansour, A.M., Kholif, A.M., 2011. The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. Int. J. Dairy Sci. 6, 267-277.

Kholif, A.E., Baza-García, L.A., Elghandour, M.M., Salem, A.Z., Barbabosa, A., Dominguez-Vara, I.A. and Sanchez-Torres, J.E., 2016. In vitro assessment of fecal inocula from horses fed on high-fiber diets with fibrolytic enzymes addition on gas, methane, and carbon dioxide productions as indicators of hindgut activity. J. Equine Vet. Sci., 39, 44-50.

Kholif, A.E., Elghandour, M.M.Y., Rodríguez, G.B., Olafadehan, O.A., Salem, A.Z.M., 2017. Anaerobic ensiling of raw agricultural waste with a fibrolytic enzyme cocktail as a cleaner and sustainable biological product. J. Clean. Prod. 142, 2649–2655.

Kumar, U., Sareen, V.K., Singh, S., 1997. Effect of yeast culture supplement on ruminal microbial populations and metabolism in buffalo calves fed a high roughage diet. J. Sci. Food Agric. 73, 231e236.

Lewis, G.E., Sanchez, W.K., Hunt, C.W., Guy, M.A., Pritchard, G.T., Swanson, B.I., Treacher R.J., 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. J. Dairy Sci. 82, 611-617.

Mao H.L., Wu C.H., Wang J.K., Liu J.X., 2013. Synergistic effect of cellulase and xylanase on in vitro rumen fermentation and microbial population with rice straw as substrate. Anim. Nutr. Feed Technol. 13, 477–487.

523 McAllister T.A., Hristov A.N., Beauchemin K.A., Rode L.M., Cheng K.-J. (2001): Enzymes
524 in ruminant diets. In: Bedford M., Partridge G. (eds.): Enzymes in Farm Animal
525 Nutrition. CABI Publishing, Wallingford, UK, 273–298.

526 Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The
527 estimation of the digestibility and metabolizable energy content of ruminant feeding
528 stuffs from the gas production when they are incubated with rumen liquor in vitro. J.
529 Agr. Sci., Cambridge., 93, 217–222

530 Morsy, T.A., Kholif, A.E., Kholif, S.M., Kholif, A.M., Sun, X., Salem, A.Z.M., 2016. Effects
531 of two enzyme feed additives on digestion and milk production in lactating Egyptian
532 buffaloes. Ann. Anim. Sci. 16, 209–222.

533 Mosoni, P., Chaucheyras-Durand, F., Berat- Maillet, C., Forano, E., 2007. Quantifica - tion
534 by real time PCR of cellulolitic bacteria in the rumen of sheeps after supplementation
535 of a forage diet with readily fermentable carbohydrates. Effect of a yeast additive. J.
536 Appl. Microbiol. 103:2676-2685.

537 Mould, F.L., Kliem, K.E., Morgan, R. and Mauricio, R.M., 2005. In vitro microbial
538 inoculum: a review of its function and properties. Anim. Feed Sci. Technol., 123, 31-
539 50.

540 Newbold, C.J., Rode, L.M., 2006. Dietary additives to control methanogenesis in the rumen.
541 Int. Congr. Ser. 1293, 138-147.

542 Newbold, C.J., Wallace, R.J., McIntosh, F.M., 1996. Mode of action of the yeast
543 *Saccharomyces cerevisiae* as a feed additive for ruminants. Br. J. Nutr. 76, 249e261.

544 Nkrumah, J.D., Okine, E.K., Mathison, G.W., Schmid, K., Li, C., Basarab, J.A., Price, M.A.,
545 Wang, Z., Moore, S.S., 2006. Relationships of feedlot feed efficiency, performance,

546 and feeding behavior with metabolic rate, methane production, and energy partitioning
547 in beef cattle. J. Anim. Sci. 84(1), 145-153.

548 Nsereko, V.L., Beauchemin, K.A., Morgavi, D.P., Rode, L.M., Furtado, A.F., McAllister,
549 T.A., Iwaasa, A.D., Yang, W.Z., Wang, Y., 2002. Effect of a fibrolytic enzyme
550 preparation from Trichoderma longibrachiatum on the rumen microbial population of
551 dairy cows. Can. J. Microbiol. 48, 14–20.

552 Patra, A.K., 2012. The use of live yeast products as microbial feed additives in ruminant
553 nutrition. Asian J. Anim. Vet. Adv. 7:366- 375.

554 Paya, H., Taghizadeh, A., Janmohammadi, H., Moghadam, G.A., 2007. Nutrient digestibility
555 and gas production of some tropical feeds used in ruminant diets estimated by the in
556 vivo and in vitro gas production techniques. Am. J. Anim. Vet. Sci. 2:108- 113.

557 Polyorach, S., Wanapat, M., Cherdthong, A., 2014. Influence of yeast fermented cassava chip
558 protein (YEFECAP) and roughage to concentrate ratio on ruminal fermentation and
559 microorganisms using in vitro gas production technique. Asian Australas. J. Anim. Sci.
560 27, 36–45.

561 Rodriguez, M.P., Mariezcurrena, M.D., Mariezcurrena, M.A., Lagunas, B.C., Elghandour,
562 M.M., Kholif, A.M., Kholif, A.E., Almaráz, E.M., Salem, A.Z., 2015. Influence of live
563 cells or cells extract of *Saccharomyces cerevisiae* on in vitro gas production of a total
564 mixed ration. Ital. J. Anim. Sci. 14(4), 590-595.

565 Salem, A.Z., Kholif, A.E., Elghandour, M.M., Buendía, G., Mariezcurrena, M.D., Hernandez,
566 S.R. and Camacho, L.M., 2014. Influence of oral administration of Salix babylonica
567 extract on milk production and composition in dairy cows. Ital. J. Anim. Sci., 13(1), 10-
568 14.

569 Salem, A.Z.M., Alsersy, H., Camacho, L.M., El-Adawy, M.M., Elghandour, M.M.Y., Kholif,
570 A.E., Rivero, N., Alonso, M.U. and Zaragoza, A., 2015. Feed intake, nutrient
571 digestibility, nitrogen utilization, and ruminal fermentation activities in sheep fed
572 Atriplex halimus ensiled with three developed enzyme cocktails. Czech J. Anim. Sci,
573 60(4), 185-194.

574 SAS, 2002. Statistical Analysis System. User's Guide: Statistics. Ver 9.0. SAS Institute,
575 Cary, NC.

576 Stewart, C.S., Flint, H.J., Bryant, M.P., 1997. The rumen bacteria. In: The Rumen Microbial
577 Ecosystem (Eds. P.N. Hobson and C.S. Stewart), Chapman and Hall, London, UK, pp.
578 10-72.

579 Valdes, K.I., Salem, A.Z.M., López, S., Alonso, M.U., Rivero, N., Elghandour, M.M.Y.,
580 Domínguez, I.A., Ronquillo, M.G., Kholif, A.E., 2015. Influence of exogenous
581 enzymes in presence of Salix babylonica extract on digestibility, microbial protein
582 synthesis and performance of lambs fed maize silage. J. Agr. Sci. 153(04), 732-742.

583 Vallejo, L.H., Salem, A.Z.M., Kholif, A.E., Elghangour, M.M.Y., Fajardo, R.C., Rivero, N.,
584 Bastida, A.Z., Mariezcurrena, M.D., 2016. Influence of cellulase or xylanase on the in
585 vitro rumen gas production and fermentation of corn stover. Indian J. Anim. Sci. 86(1),
586 70-74.

587 Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fibre, neutral
588 detergent fibre, and non-starch carbohydrates in relation to animal nutrition. J. Dairy
589 Sci. 74, 3583–3597.

590 Velázquez-Garduño, G., Mariezcurrena-Berasain, M.A., Salem, A.Z., Gutiérrez-Ibañez, A.T.,
591 Bernal-Martínez, L.R., Pinzón-Martínez, D.L., Kholif, A.E., Odongo, N.E.,
592 Mariezcurrena-Berasain, M.D., 2015. Effect of organic selenium-enriched yeast

593 supplementation in finishing sheep diet on carcasses microbiological contamination and
594 meat physical characteristics. Ital. J. Anim. Sci. 14(3), p.3836.

595 Williams, P.E.V., Tait, C.A.G., Innes, G.M., Newbold, C.J., 1991. Effects of the inclusion of
596 yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of cows on
597 milk yield and forage degradation and fermentation patterns in the rumen of sheep and
598 steers. J. Anim. Sci. 69:3016- 3026.

599

Table 1

Biogases production (mL/g DM) of a total mixed ration as affected by the addition of xylanase, *S. cerevisiae* yeast and their mixture, and rumen liquor from goats, sheep and steers

Inoculum	Additive	Gas production parameters				CH_4 production at 48 h of incubation			CO_2 production at 48 h of incubation		
		b	*c*	*Lag*	mL gas/g degraded DM	mL CH_4/g incubated DM	Proportional CH_4 production	mL CH_4/g degraded DM	CO_2 production (mL/g DM)^2	Proportional CO_2 production	mL CO_2/g degraded DM
Goat	Control	270	0.062	4.91	357	246.7	87.1	349.2	36.4	12.9	51.5
	Xylanase	278	0.069	4.76	369	242.6	83.9	337.0	46.5	16.1	64.6
	Yeast	288	0.066	4.22	366	241.0	83.0	339.1	49.5	17.0	69.6
	Xylanase+yeast	267	0.069	4.83	352	242.9	83.2	335.7	48.9	16.8	67.6
	SEM	5.1	0.0021	0.187	6.0	1.3	0.40	2.7	0.86	0.31	0.62
	P-value	0.379	0.048	0.107	0.236	0.051	0.046	0.041	0.012	0.048	0.037
Sheep	Control	274	0.059	4.40	365	222.9	87.1	311.2	33.1	12.9	46.2
	Xylanase	281	0.064	4.32	367	219.9	82.3	303.7	47.3	17.7	65.4
	Yeast	283	0.062	4.04	374	213.9	81.3	297.6	49.3	18.7	68.5
	Xylanase+yeast	290	0.059	3.59	373	214.2	83.1	292.5	43.4	16.9	59.3
	SEM	6.5	0.0031	0.473	8.3	1.4	0.52	1.9	0.33	0.62	1.19
	P-value	0.045	0.046	0.634	0.854	0.760	0.040	0.024	0.048	0.047	0.019
Steers	Control	292	0.073	5.15	401	221.6	86.0	313.8	36.2	14.0	51.3

	Xylanase	297	0.075	4.54	402	222.5	83.2	305.7	44.9	16.8	61.7
	Yeast	300	0.073	4.41	409	224.4	83.6	312.4	44.0	16.4	61.3
	Xylanase+yeast	303	0.070	4.38	403	227.1	83.6	311.7	44.6	16.4	61.2
	SEM	6.5	0.0029	0.299	8.3	1.6	0.49	1.80	1.19	1.49	1.60
	P-value	0.677	0.595	0.029	0.907	0.404	0.041	0.605	0.069	0.055	0.007
Pooled SEM		6.1	0.0027	0.341	7.622	2.50	0.82	2.6	1.52	0.82	1.57
P value											
Inoculum		<0.001	<0.001	0.041	<0.001	<0.001	0.049	<0.001	0.027	0.527	0.018
Additive		0.016	0.024	0.014	0.547	0.012	0.045	0.621	0.887	0.959	0.872
Inoculum × additive		0.694	0.434	0.750	0.843	0.215	0.143	0.229	0.403	0.138	0.449

[2] *b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *Lag* is the initial delay before gas production begins (h).

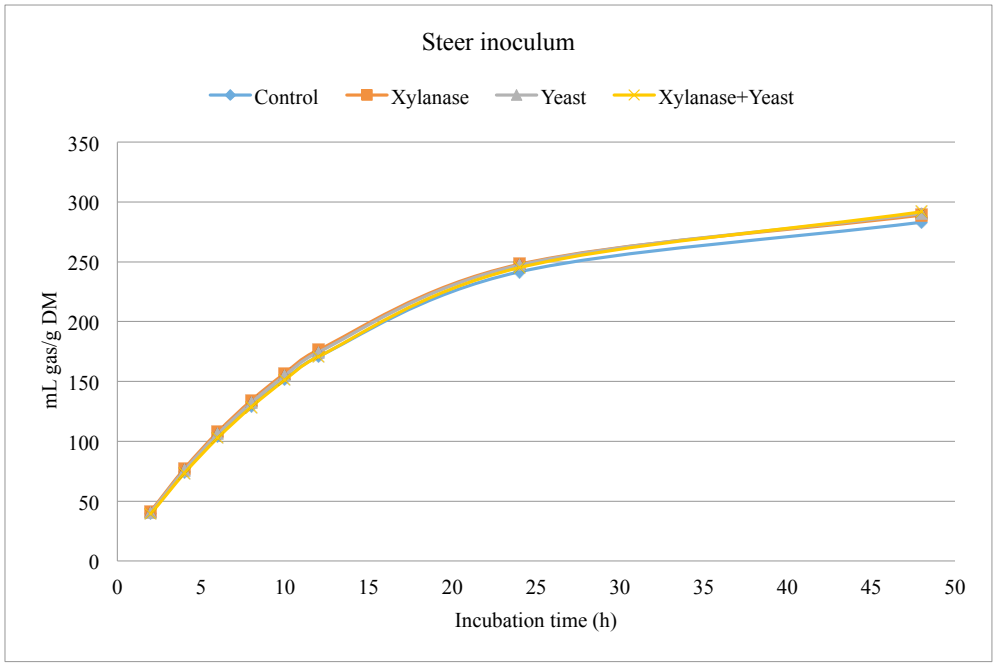
Table 2

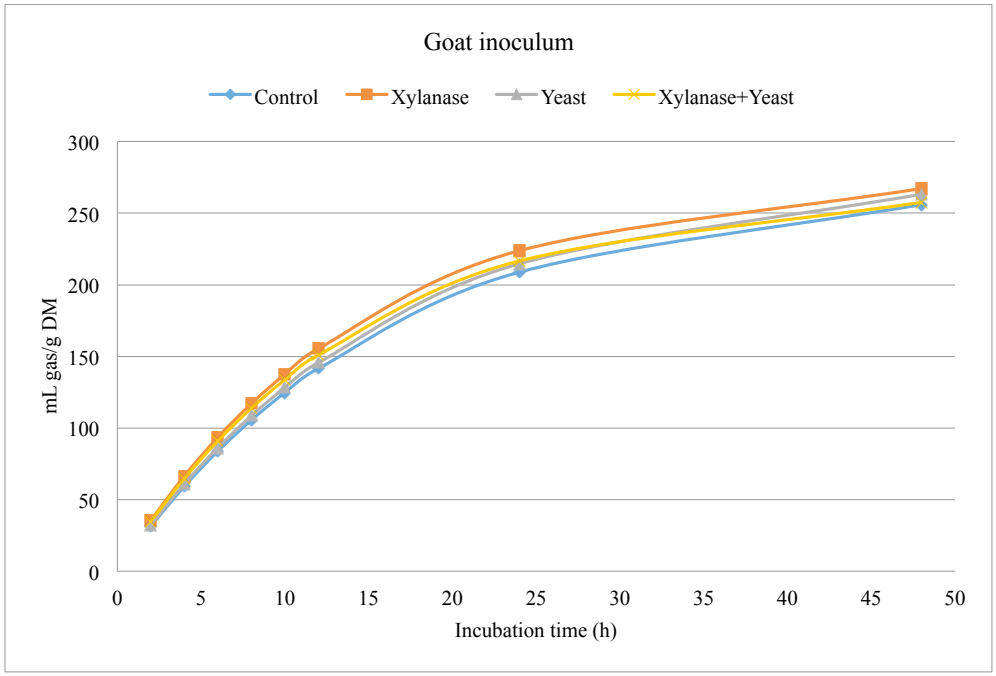
Fermentation kinetics of a total mixed ration as affected by the addition of xylanase, *S. cerevisiae* yeast and their mixture, and rumen liquor from goats, sheep and steers

Inoculum	Additive	pH	SCFA	NH_3-N	DMD	OMD	ME	PF_{24}	GY_{24}	MCP	Total bacteria × 10^8	Total protozoa × 10^5
Goat	Control	6.50	4.62	66.2	716	605	8.92	5.39	185	667	7.0	2.03
	Xylanase	6.50	4.95	65.3	724	632	9.33	5.30	189	695	11.6	2.65
	Yeast	6.49	4.74	64.7	719	615	9.08	5.35	187	677	11.3	2.79
	Xylanase+yeast	6.51	4.79	66.7	733	619	9.14	5.35	187	681	11.5	2.83
	SEM	0.020	0.076	2.02	6.0	2.1	0.093	0.022	0.7	1.4	0.25	0.417
	P-value	0.821	0.041	0.895	0.289	0.048	0.019	0.102	0.091	0.042	0.028	0.510
Sheep	Control	6.50	4.59	55.0	706	603	8.89	5.40	185	664	8.4	2.77
	Xylanase	6.52	4.86	55.2	728	625	9.23	5.33	188	687	10.9	2.30
	Yeast	6.48	4.84	54.4	719	623	9.20	5.33	188	685	11.4	2.32
	Xylanase+yeast	6.49	4.83	59.7	729	622	9.19	5.34	188	685	11.9	2.29
	SEM	0.013	0.130	1.29	3.7	10.5	0.160	0.034	1.2	11.0	0.42	0.289
	P-value	0.279	0.045	0.068	0.009	0.043	0.046	0.457	0.459	0.457	0.045	0.446
Steers	Control	6.50	5.34	62.2	707	663	9.82	5.21	192	728	7.9	2.89
	Xylanase	6.48	5.49	62.5	720	674	9.99	5.18	193	740	11.1	2.23
	Yeast	6.48	5.47	57.3	711	673	9.97	5.19	193	738	10.8	2.28
	Xylanase+yeast	6.49	5.42	60.7	724	670	9.91	5.20	192	735	11.5	2.16
	SEM	0.003	0.079	1.33	3.7	6.3	0.096	0.016	0.6	6.6	0.45	0.435
	P-value	0.222	0.597	0.085	0.037	0.624	0.604	0.556	0.625	0.602	0.044	0.170

Pooled SEM	0.014	0.098	1.59	4.6	7.9	0.120	0.025	0.9	8.3	0.38	0.215
P value											
Inoculum	0.545	<0.001	<0.001	0.076	<0.001	<0.001	<0.001	<0.001	<0.001	0.167	0.002
Additive	0.289	0.035	0.075	0.002	0.034	0.035	0.033	0.038	0.035	0.368	0.096
Inoculum × additive	0.661	0.907	0.363	0.843	0.905	0.907	0.865	0.861	0.909	0.649	<0.001

[1]DMD is dry matter degradability (mg/g DM), GY_{24} is gas yield at 24 h (mL gas/g DMD), MCP is microbial protein production (mg/g DM), ME is metabolizable energy (MJ/kg DM), NH_3-N is ammonia-N, OMD is in vitro organic matter digestibility (g/kg DM), PF_{24} is partitioning factor at 24 h of incubation (mg DMD/mL gas), pH is ruminal pH, SCFA is short-chain fatty acids (mmol/g DM).


Steer inoculum
Control
Xylanase
Yeast
Xylanase+Yeast
mL gas/g DM
0
50
100
150
200
250
300
350
0
5
10
15
20
25
30
35
40
45
50
Incubation time (h)


Goat inoculum
Control
Xylanase
Yeast
Xylanase+Yeast
mL gas/g DM
0
50
100
150
200
250
300
0
5
10
15
20
25
30
35
40
45
50
Incubation time (h)

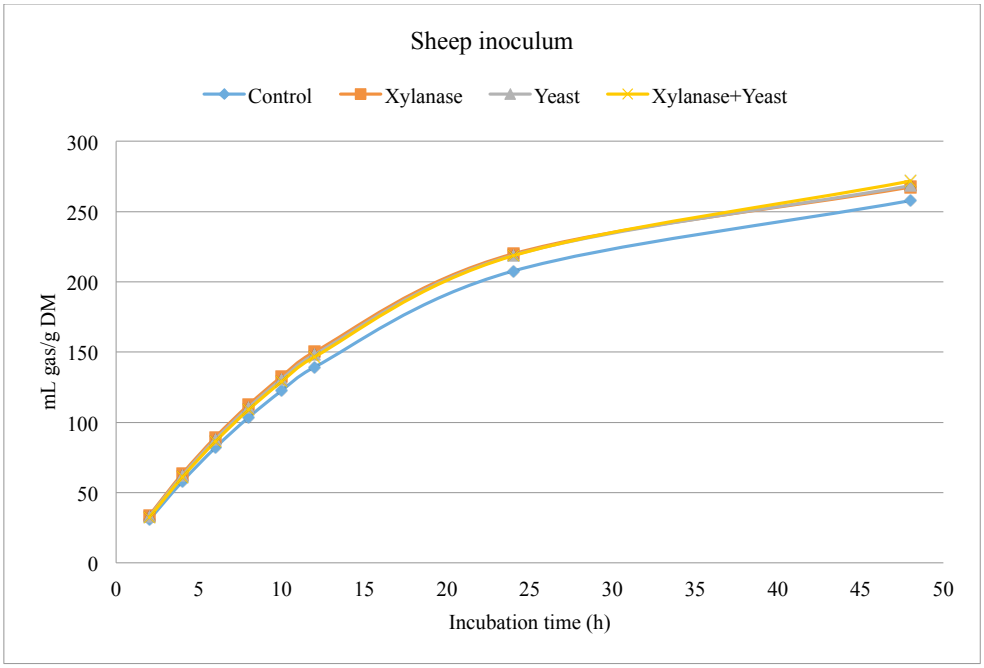


Fig. 1

Gas production (mL/g DM) as affected by the inclusion of *S. cerevisiae*, xylanase, and their mixture, and with the using of rumen inoculum from goats, sheep, and steers

VIII. DISCUSIÓN GENERAL

Se estudió el uso de aditivos fibrolíticos en dietas para rumiantes, en específico celulasa, xilanasa y levadura *de Saccharomyces cerevisiae*, y su efecto sobre algunas variables de la cinética fermentación ruminal, producción de gas y degradabilidad de un sustrato fibroso *in vitro*, así como la digestibilidad de nutrientes *in vivo*.

En la prueba *in situ* la adición de la dosis alta de xilanasa (XY6) disminuyó el consumo de alimento, además la digestibilidad total en el tracto se incrementó (28-42%). Esto sugiere que la capacidad enzimática fue la óptima, lo que podría mejorar la eficiencia de fermentación durante las etapas iniciales de la digestión (Jalilvand *et al*., 2008), considerando que un descenso en el pH indica mayor hidrólisis del sustrato, el tratamiento de XY6 mostró menor valor de pH a la hora 3 postprandial, aunado a esto se observa mayor concentración de ácido acético, lo cual puede asociarse con una mejor digestión de carbohidratos estructurales (Soltan *et al*., 2013) modificando la estructura de las fibras, lo que puede estimular la adhesión y colonización bacteriana (Giraldo *et al.,* 2008), así como las interacciones complementarias con las enzimas microbianas ruminales. Las mayores concentraciones de amoníaco ruminal en las ovejas alimentadas con dietas tratadas con enzimas (XY6 y XY3) en comparación con el control sin suplementar apoyan la posibilidad de que la xilanasa realce la degradación de la proteína ruminal, probablemente en respuesta a un cambio en la microbiota ruminal (Salem *et al.,* 2013).

En la segunda prueba, se evaluaron, *in vitro,* dosis crecientes de enzimas celulasas y xilanasas adicionadas a rastrojo de maíz, el cual fue incubado con líquido ruminal, se evaluó la producción de gas, la cinética de fermentación y la degradabilidad de la parte fibrosa del sustrato. La tasa de producción de gas no fue afectada por el tipo o dosis de enzima, sin embargo, se observan diferencias en la fracción L (fase lag) cuando se adicionó celulasa, lo cual puede ser atribuible a que la celulosa es un biopolímero con enlaces que son degradados rápidamente, lo que se traduce en mayor producción de gas a menor tiempo, en

contraste, cuando se adicionó xilanasa, el efecto fue a la fracción *b* (producción de gas asintótica), esto puede deberse a que la acción enzimática sigue degradando las paredes celulares, cuando se aplicó la dosis de 80 µg/g MS, la curva de producción de gas alcanzó su punto b a los 240.5 mL/g MS de gas producido, mientras que las demás dosis se mantuvieron aproximadamente en 218 mL/g MS; sin embargo la producción de gas total con este nivel de inclusión afecto negativamente la fermentación, es probable que los niveles de XYL impidieran la unión de la enzimas a los receptores del sustrato, limitando también la adhesión bacteriana las partículas de alimento (Treacher y Hunt, 1996). La adición de ambas enzimas disminuyó el pH del medio, lo cual concuerda con la hipótesis de que existe una mayor hidrólisis enzimática del sustrato fermentado y los metabolitos resultantes disminuyen el pH del medio, este supuesto es reforzado con lo observado con AGV y proteína microbiana, ya que se observa una relación positiva entre la dosis de enzima y la concentración final de AGV y PMC. Es por ello que la cantidad de materia orgánica degradada que es mayor cuando se adicionó las enzimas.

En la última prueba donde se evaluó el efecto de xilanasa y *S. cerevisiae in vitro* los aditivos probados aumentaron la tasa de PG y disminuyeron el tiempo de retraso de PG lo que reveló una mejor utilización de nutrientes. Estudios recientes mostraron que la inclusión de enzimas exógenas en dietas de rumiantes mejoró la utilización del alimento, la digestión de la MS y el rendimiento animal mejorando la degradación de la MS (Morsy *et al.,* 2016). Rodriguez *et al.* (2015) observaron que la adición de *S. cerevisiae* disminuyó la tasa de PG. La inconstancia puede deberse a la composición del sustrato (Elghandour et al., 2014). El menor tiempo de retardo de PG puede deberse a una mayor degradación de los nutrientes del alimento, especialmente las fibras (Kholif *et al*., 2016, Elghandour *et al.,* 2017). Las enzimas exógenas tienen la capacidad de estimular las fases iniciales de colonización microbiana en el rumen y de facilitar la fijación bacteriana a las partículas de alimento (Giraldo *et al.,* 2007). Además, Callaway y Martin (1997) informaron que *S. cerevisiae* contiene pequeños péptidos y nutrientes importantes requeridos para el crecimiento y la actividad de los microorganismos ruminales,

especialmente las bacterias celulolíticas ruminales para iniciar el proceso de fermentación. La inclusión de *S. cerevisiae* en las dietas de los rumiantes demostró aumentar la digestibilidad de nutrientes (Hassan *et al.,* 2016) y alterar la producción de AGV en el rumen elevando las poblaciones de bacterias celulolíticas y amilolíticas (Kumar *et al.* 1997).

Una de las limitantes en el uso de aditivos fibrolíticos en dietas para rumiantes es el costo final por inclusión de enzimas o levaduras, el argumento radica en que el costo de alimentación no se ve compensado con mejoras constantes en las variables de producción animal, principalmente en GDP, CA, peso final o rendimiento de la canal y producción de leche, Ortíz-Rodea *et al* (2013) realizaron un meta-análisis para evaluar el efecto de la adición de enzimas exógenas en la alimentación de vacas lecheras, reportan que los parámetros de producción de leche ($P>0.16$) y sus componentes, grasa ($P=0.88$), proteína ($P=0.39$) o lactosa ($P=0.95$), no fueron afectados por la administración de enzimas exógenas; además de bajos coeficientes de determinación entre la administración de la enzima (g/KgPV$^{0.75}$) y la producción de leche (g/KgPV$^{0.75}$) (R^2 0.001), el contenido de grasa (g/100g leche) (R^2 0.007), lactosa (g/100g leche) (R^2 0.120) y proteína (g/100g leche) (R^2 0.172), aunado a esto, la respuesta a las enzimas puede modificarse en función del sustrato (Yescas *et al.,* 2004). En la presente investigación, no fue posible realizar un análisis de costo-beneficio, pues no se llevó a cabo una prueba de comportamiento productivo. Sin embargo, se observan incrementos en algunas variables de fermentación ruminal y degradación del sustrato, lo cual indica una mejora visible de la función ruminal y podría esperarse, en dado caso, incremento en la energía disponible para el rumiante y por lo tanto, incremento en los valores de los parámetros productivos.

IX. CONCLUSIONES

La adición de enzimas fibrolíticas en dietas con rastrojo de maíz para rumiantes disminuyo el pH, aumento las concentraciones de N-NH_3 y AGV *in vitro* e *in situ*, además aumentó la digestibilidad aparente de nutrientes *in vivo*.

Con las dosis de 3 y 6 µL de xilanasa/g de MS aplicadas a una dieta con 30% de rastrojo de maíz para ovinos, se observó mayor digestibilidad aparente y un impacto positivo en la fermentación ruminal, sin afectar las concentraciones séricas de urea, fósforo y triglicéridos de borregos Rambouillet.

La adición de enzimas exógenas: celulasa y xilanasa, aumentó la producción de gas *in vitro* y la cinética de fermentación del rastrojo de maíz. Siendo la dosis de 40 µg/gMS la que mayor efecto reflejó.

La combinación de enzima xilanasa con levadura de *S. cerevisiae* no afectó la producción de gas *in vitro,* la degradabilidad, ni la concentración de microorganismos ruminales. Sin embargo, se observó una disminución en la producción de CH_4. Las variables de fermentación ruminal *in vitro* fueron diferentes entre bovinos, ovinos y caprinos.

X. LITERATURA CITADA

Abdel-Aziz, N.A., Salem, A.Z.M., El-Adawy, M.M., Camacho, L.M., Kholif, A.E., Elghandour, M. M.Y. and Borhami, B.E. 2015. Biological treatments as a mean to improve feed utilization in agriculture animals-An overview. J. Integr. Agri. 14:534–543.

Aderinboye, R.Y., Akinlolu, A.O., Adeleke, M.A., Najeem, G.O., Ojo, V.O.A., Isah, O.A. and Babayemi, O.J., 2016. *In vitro* gas production and dry matter degradation of four browse leaves using cattle, sheep and goat inocula. Slovak J. Anim. Sci. 49:32-43.

Ahmed, M.H., Elghandour, M.M.Y., Salem, A.Z.M., Zeweil, H.S., Kholif, A.E., Klieve, A.V., Abdelrassol, A.M.A., 2015. Influence of *Trichoderma reesei* or *Saccharomyces cerevisiae* on performance, ruminal fermentation, carcass characteristics and blood biochemistry of lambs fed Atriplex nummularia and Acacia saligna mixture. Livest. Sci. 180:90-97.

Almaraz, I., González, S.S., Pinos-Rodríguez, J.M. and Miranda, L.A. 2010. Effects of exogenous fibrolytic enzymes on *in sacco* and *in vitro* degradation of diets and on growth performance. Ital. J. Ani. Sci. 9:6-10.

Alsersy, H., Salem, A.Z.M., Borhami, B.E., Olivares, J., Gado, H.M., Mariezcurrena, M.D., Yacuot, M.H., Kholif, A.E., El-Adawy, M. and Hernández, S.R. 2015. Effect of Mediterranean saltbush (*Atriplex halimus*) ensilaging with two developed enzyme cocktails on feed intake, nutrient digestibility and ruminal fermentation in sheep. Anim. Sci. J., 86:51–58.

Aman, P. 1993. Composition and structure of cell wall polysaccharides in forages. *In*: Forage cell wall structure and digestibility. Jung H.G.; Buxton R.D., Hatfield, R.D., Ralph, J. (Eds) ASA-CSSA-SSSA, Madison, Wisconsin, USA, pp: 183-199.

Ammar, H., Ranilla, M.J., Tejido, M.L., Ovejero, F.J., González, J.S. and López, S. 2004. Effect of inoculum source (sheep or goat rumen fluid) on *in vitro*

digestibility and gas production kinetics of the foliage of some Spanish browse plants. Options Mediterraneennes. Serie A, Seminaires Mediterraneenes. Centre International de Hautes Etudes Agronomiques Mediterraneens, Montpellier, France. 59, pp. 121–126.

Arce-Cervantes, O., Mendoza, G.D., Hernández, P.A., Meneses, M., Torres-Salado, N. and Loera, O. 2013. The effect of a lignocellulolytic extract of *Fomes sp*. EUM1 on the intake, digestibility, feed efficiency and growth lambs. Anim. Nutr. Feed Technol. 13:363-372.

Association of Official Analytical Chemists. 1997. Official Methods of Analysis, 16th ed. AOAC, Arlington, VA, USA.

Association of Official Analytical Chemists. 2003. Official methods of analysis of AOAC (Association of Official Analytical Chemists) International (17th ed.). AOAC International, Gaithersburg, MD.

Baumann, T.A., Radunz, A.E., Lardy, G.P., Anderson, V.L., Caton, J.S. and Bauer, L. 2004. Effects of tempering and yeast-enzyme mixture on intake, ruminal fermentation, *in situ* disappearance, performance, and carcass traits in steers fed barley-based diets. Prof. Anim. Sci. 20:178-184.

Beauchemin, K. A., Colombatto, D., Morgavi, D. P., and Yang, W. Z. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81:E37–E47.

Beauchemin, K. A., Colombatto, D., Morgavi, D. P., Yang, W. Z. and Rode, L. M. 2004. Mode of action of exogenous cell wall degrading enzymes for ruminants. Can. J. Anim. Sci. 84:13–22.

Beauchemin, K.A. and Holtshausen, L. 2010. Developments in enzyme usage in ruminants. *In:* Enzymes in Farm Animal Nutrition. Bedford, M.R. and Partridge,G.G.(Eds). 2nd edn, CABI, Oxford, United Kingdom, pp. 206-230.

Blümmel, M., Steingss, H., Becker, K., 1997. The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and ^{15}N incorporation and its implications for the prediction of voluntary feed intake of roughages. Br. J. Nutr. 77, 911–921.

Bodas, R., Prieto, N., García-González, R., Andrés, S., Giráldez, F.J. and López, S. 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim. Feed Sci. Technol. 176:78-93.

Boeckaert, C., Vlaeminck, B., Mestdagh, J. and Fievez, V., 2007. *In vitro* examination of DHA-edible micro algae: 1. Effect on rumen lipolysis and biohydrogenation of linoleic and linolenic acids. Anim. Feed Sci. Technol., 136(1):63-79.

Boyd, J.W. 2011. The interpretation of serum biochemistry test results in domestic animals. *In:* Veterinary Clinical Pathology, Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc.

Broderick, G.A. and Kang, J.H. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy Sci. 63:64–75.

Bruinenberg, M. H., Van Der Honing, Y., Agnew, R. E., Yan, T., Van Vuuren, A. M. and Valk, H. 2002. Energy metabolism of dairy cows fed on grass. Livest. Prod. Sci. 75:117-128.

Bueno, I., Abdalla, A., Cabral Filho, S.L.S., Vitti, D., Owen, E., Mauricio, R., Givens, I., Sutton, J. And Mould, F., 1999. Comparison of inocula from sheep and cattle for the *in vitro* gas production under tropical conditions. In: Annual Meeting of The British Society of Animal Science. 13:151-156.

Callaway, E.S. and Martin, S.A., 1997. Effects of a *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

Carro, M.D., Ranilla, M.J. y Tejido, M.L. 2006. Utilización de aditivos en la alimentación de ganado ovino y caprino. XXXI Jornadas Científicas de la SEOC, Zamora, pp 26-37.

Cayetano, J.A., Salem, A.Z.M., Mariezcurrena, B.M.A., Rojo, R., Cerillo-Soto, M.A., Gado, H. and Camacho, L.M. 2013. Effect of adding *Salix babylonica* extracts and exogenous enzymes to basal diets on the meat quality of growing Suffolk lambs. Anim Nutr Feed Technol. 13:373-380.

Chung Y.H., Zhou M., Holtshausen L., Alexander T.W., McAllister T.A., Guan L.L., Oba M., Beauchemin K.A. 2012. A fibrolytic enzyme additive for lactating Holstein cow diets: ruminal fermentation, rumen microbial populations, and enteric methane emissions. J. Dairy Sci. 95:1419–1427.

Church, D.C. 1993. El Rumiante. Fisiología digestiva y nutrición. Acriba, Zaragoza, 641 p.

Colombatto, D., Mould, F.L., Bhat, M.K., Morgavi, D.P., Beauchemin, K.A. and Owen, E. 2003. Influence of fibrolytic enzymes on the hydrolysis and fermentation of pure cellulose and xylan by mixed ruminal microorganisms *in vitro*. J. Anim. Sci., 81:1040–1050.

Corona, L., Mendoza, G.D., Castrejón, F.A., Crosby, M.M. and Cobos, M.A., 1999. Evaluation of two yeast cultures (*Saccharomyces cerevisiae*) on ruminal fermentation and digestion in sheep fed a corn stover diet. Small Rumin. Res. 31(3):209-214.

Cosgrove, D. J. 1977. Microbial transformations in the phosphorus cycle. Adv. Microb. Ecol. 1:95–134.

Dean, D.B., Valencia, E., Krueger, N.A. and Adesogan, A.T. 2013a. Effect of treatment with fibrolytic enzymes or ammonia on the nutritive value of guineagrass (*Panicum maximum*) hay. Anim. Nutr. Feed Technol. 13:517-525.

Dean, D.B., Staples, C.R., Littell, R.C., Kim, S. and Adesogan, A.T. 2013b. Effect of method of adding a fibrolytic enzyme to dairy cow diets on feed intake digestibility, milk production, ruminal fermentation, and blood metabolites. Anim. Nutr. Feed Technol. 13:337-353.

Devillard, E., Bera-Maillet, C., Flint, H.J., Scott, K.P., Newbold, C.J., Wallace, R.J., Jouany, J.P. and Forano, E. 2003. Characterization of XYN10B, a modular xylanase from the ruminal protozoan *Polyplastron multivesiculatum*, with a family 22 carbohydrate-binding module that binds to cellulose. Biochem. J. 373:495-503.

Díaz, A., Carro, M.D., Saro, C., Mateos, I., Odongo, E. and Ranilla, M.J. 2013. *In vitro* evaluation of commercial fibrolytic enzymes for improving the nutritive value of low-quality forages. Anim. Nutr. Feed Technol., 13:461-476.

Elghandour, M.M.Y., Salem, A.Z.M., Gonzalez-Ronquillo, M., Brquez, J.L., Gado, H.M., Odongo, N.E. and Penuelas, C.G. 2013. Effects of exogenous enzymes on *in vitro* gas production kinetics and ruminal fermentation of four fibrous feeds. Anim. Feed Sci. Technol., 179:46– 53.

Elghandour, M.M., Chagoyán, J.C.V., Salem, A.Z., Kholif, A.E., Castañeda, J.S.M., Camacho, L.M. and Cerrillo-Soto, M.A., 2014. Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on *in vitro* gas production kinetics and degradability of four fibrous feeds. Ital. J. Anim. Sci. 13(2):295-301.

Elghandour, M.M.Y., Salem, A.Z.M., Martínez Castañeda, J.S., Camacho, L.M., Kholif, A.E. and Vázquez Chagoyán, J.C. 2015. Direct-fed microbes: A tool for improving the utilization of low quality roughages in ruminants. J. Integr. Agr., 14:526–533.

Elghandour, M.M.M.Y., Kholif, A.E., Marquez-Molina, O., Vazquez-Armijo, J.F., Puniya, A.K. and Salem, A.Z.M., 2015. Influence of individual or mixed cellulase and xylanase mixture on *in vitro* rumen gas production kinetics of

total mixed rations with different maize silage and concentrate ratios. Turk. J. Vet. Anim. Sci. 39(4):435-442.

Elghandour, M.M.Y., Kholif, A.E., Salem, A.Z.M., de Oca, R.M., Barbabosa, A., Mariezcurrena, M. and Olafadehan, O.A., 2016a. Addressing sustainable ruminal methane and carbon dioxide emissions of soybean hulls by organic acid salts. J. Clean. Prod. 135:194–200.

Elghandour M.M.Y., Kholif, A.E., Hernández, J., Mariezcurrena, M.D., López, S., Camacho, L.M., Márquez, O., Salem, A.Z.M., 2016b. Influence of the addition of exogenous xylanase with or without pre-incubation on the *in vitro* ruminal fermentation of three fibrous feeds. Czech J. Anim. Sci. 61 (6):262–272.

Elghandour, M.M.Y., Vázquez Chagoyán, J.C., Salem, A.Z.M., Kholif, A.E., Martínez Castañeda, J.S., Camacho, L.M. and Cerrillo-Soto, M.A. 2014. Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on *in vitro* gas production kinetics and degradability of four fibrous feeds. Ital. J. Anim. Sci., 13:295-301.

Elghandour, M.M.Y., Vázquez, J.C., Salem, A.Z.M., Kholif, A.E., Cipriano, M.M., Camacho, L.M., Márquez, O., 2017. *In vitro* gas and methane production of two mixed rations influenced by three different cultures of *Saccharomyces cerevisiae*. J. Appl. Anim. Res. 45:389-395.

Elwakeel, E.A., Titgemeyer, E.C., Johnson, B.J., Armendariz, C.K. and Shirley, J.E. 2007. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. J. Dairy Sci. 90:5226–5236.

Erwin E. S., Marco G. J., and Emery, E. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768–1771.

FAO (Food and Agriculture Organization of the United Nations), 2006. Livestock a Major Threat to the Environment: Remedies Urgently Needed. [accessed 26

December 2016]. Available:http://www.fao.org/newsroom/en/news/2006/1000448/index.html.

Fedorak, P.M. and Hrudey, S.E. 1983. A simple apparatus for measuring gas production by methanogenic cultures in serum bottles. Envir. Tecnhol. Lett. 425-432.

Fonty, G. and Chaucheyras, F.D. 2006.Effects and modes of action of live yeasts in the rumen. Biología 61:741-750.

Forsberg, C.W., Cheng, K.J. and White, B.A. 1997. Polysaccharide degradation in the rumen and large intestine. In: Gastrointestinal Microbiology. Mackie, R.I. and White, B.A. Eds. Chapman and Hall, New York. pp-319-379.

Forsberg, C., Forano, E. and Chesson, A. 2000. Microbial adherence to the plant cellwall and enzymatic hydrolysis. In: Cronje, P.B. (Ed.), Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. CABI Publishing, Wallingford, UK, pp. 79–97.

France, J., Dijkstra, J., Dhanoa, M.S., López, S. and Bannink, A. 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. Br. J. Nutr. 83:143–150.

Gado, H.M., Salem, A.Z.M., Robinson, P.H. and Hassan, M. 2009. Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Anim. Feed Sci. Technol. 154:36–46.

Gado, H. and Salem, A.Z.M. 2013a. Anaerobic enzymes as a new technology in animal feed. *In:* Nutritional strategies of animal feed additives. Salem, A.Z.M. (Ed). Nova Science Publishers. New York, USA. pp. 1-24.

Gado, H., Salem, A.Z.M., Camacho, L.M., Elghandour, M.M.Y. and Salazar, M.C. 2013b. Influence of exogenous enzymes on *in vitro* ruminal degradation of ensiled rice straw with DDGS. Anim. Nutr. Feed Technol. 13:569-574.

García, E. 1981. Modificaciones al sistema de clasificación climática de Köppen para Adaptarlo a las Condiciones de la República Mexicana. Offset Larios. México. 246 p.

García, V., Vasquez, H., Fonseca, F., Manzanares, P., Viana, F., Martínez, C and Ganga, M.A. 2010. Effects of using mixed wine yeast cultures in the production of Chardonnay wines. Rev Argent Microbiol 42(3):226-9

Getachew, G., Makkar, H.P.S., Becker, K., 2002. Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. J. Agr. Sci., 139:341–352.

Giraldo, L.A., Ranilla, M.J., Tejido, M.L. and Carro, M.D. 2004. Effect of enzyme application method on In vitro rumen fermentation of tropical forages. J. Anim. Feed Sci., 13:63–66.

Giraldo, L.A., Tejido, M.L., Ranilla, M.J., Carro, M.D., 2007. Effects of exogenous cellulase supplementation on microbial growth and ruminal fermentation of a high-forage diet in Rusitec fermenters. J. Anim. Sci. 85, 1962–1970.

Giraldo, L.A., Tejido, M.L., Ranilla, M.J., Carro, M.D. 2008. Influence of direct-fed fibrolityc enzymes on diet digestibility and ruminal activity in sheep fed a grass hay-based diet. J. Anim. Sci. 86:1617-1623.

Goering, M.K., Van Soest, P.J., 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications). Agriculture Handbook, No 379. Agricultural Research Service, USDA, Washington, DC.

Hassan, A.A., Salem, A.Z.M., Kholif, A.E., Samir, M., Yacout, M.H., Hafsa, S.A., Mendoza, G.D., Elghandour, M.M.Y., Ayala, M., Lopez, S., 2016.

Performance of crossbred dairy Friesian calves fed two levels of *Saccharomyces cerevisiae*: intake, digestion, ruminal fermentation, blood parameters and fecal pathogenic bacteria. J. Agr. Sci. 154:1488-1498.

He, Z.Y., Yang, L.Y., Yang, W.Z., Beauchemin, K.A., Tang, S.X., Huang, J.Y., Zhou, C.S., Han, X.F., Wang, M., Kang, J.H., Odongo, N.E. and Tan, Z.L. 2015. Efficacy of exogenous xylanases for improving *in vitro* fermentation of forages. J. Agr. Sci. 153:538-553.

Hernández, A., Kholif, A.E., Lugo-Coyote, R., Elghandour, M.M.Y., Cipriano, M., Rodríguez, G.B., Odongo, N.E., Salem, A.Z.M., 2017. The effect of garlic oil, xylanase enzyme and yeast on biomethane and carbon dioxide production from 60-d old Holstein dairy calves fed a high concentrate diet. J. Clean. Prod. 142:2384–2392.

Hook, S.E., Wright, A.D.G., McBride, B.W., 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea, 2010. Article ID 945785, 11 pages; doi:10.1155/2010/945785.

Hristov, A.N., Mcallister, T.A. and Cheng, K.-J. 1998. Stability of exogenous polysaccharide degrading enzyme in the rumen. J. Anim. Feed Sci. Technol. 76:165-172.

Hristov, A.N., Mcallister, T.A. and Cheng, K.-J. 2000. Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: effects on nutrient digestion in cattle fed a barley grain diet. J. Anim. Sci. 78:477-87.

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P., Adesogan, A.T., Yang, W., Lee, C., Gerber, P.J., Henderson, B., Tricarico, J.M., 2013. Special topics: mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045–5069.

Hristov, A. N., Oh, J., Giallongo, F., Frederick, T. W., Harper, M. T., Weeks, H. L., Branco, A.F., Moate, P.J., Deighton, M. H., Williams, S.R.O., Kindermann, M., Duval, S., 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proc. Nat. Acad. Sci. USA. 112(34):10663–10668.

Hungate, R.E. 1984. Microbes of nutriotional importance in the alimentary tract. Proc. Nutr. Soc. 43:1-11.

Jackson, S. and Nicolson, S. W. 2002. Xylose as a nectar sugar: From biochemistry to ecology. Comparative Biochemistry and Physiology Part B. Biochemistry and Molecular Biology 131:613–620.

Jalilvand, G., Odongo, N.E., López, S., Naserian, A., Valizadeh, R., Eftekhar, S.F., Kebreab, E. and France, J. 2008. Effects of different levels of an enzyme mixture on *in vitro* gas production parameters of contrasting forages. Anim. Feed Sci. Techonol. 146:289-301.

Jespersen L. 2003. Ocurrence and taxonomic characteristics of strains of *Saccharomyces cerevisiae* predominant in African indigenuos fermented foods and bevereages. Yest Research. 3:191-200.

Jung, H.G., D.R. Mertens, and A.J. Payne. 1997. Correlation of acid detergent lignin and Klason lignin with digestibility of forage dry matter and neutral detergent fiber. J. Dairy Sci. 80:1622-1628.

Jung, H.G., M.A. Jorgensen, J.G. Linn, and F.M. Engels. 2000. Impact of accessibility and chemical composition on cell-wall polysaccharide degradability of maize and lucerne stems. J. Sci. Food Agric. 80:419-427.

Khattab, H.M., Gado, H.M., Kholif, A.E., Mansour, A.M. and Kholif, A.M. 2011. The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. Inter. J. Dairy Sci., 6:267-277.

Khattab, H.M., Gado, H.M., Kholif, A.E., Mansour, A.M., Kholif, A.M., 2011. The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. Int. J. Dairy Sci. 6:267-277.

Khattab, H.M., Gado, H.M., Salem, A.Z.M., Camacho, L.M., El-Sayed, M.M., Kholif, A.M., Elshewy, A.A. and Kholif, A.E. (2013). Chemical Composition and *in vitro* digestibility of *Pleurotus ostreatus* spent rice straw. Anim. Nutr. Feed Technol., 13:507-516.

Kholif, A.E., Khattab H.M., El-Shewy, A.A., Salem, A.Z.M., Kholif, A.M., El-Sayed, M.M., Gado, H.M. and Mariezcurrena, M.D. 2014. Nutrient digestibility, ruminal fermentation activities, serum parameters and milk production and composition of lactating goats fed diets containing rice straw treated with Pleurotus ostreatus. Asian Australas. J. Anim. Sci., 27:357-364.

Kholif, A.M. and Aziz, H.A. 2014. Influence of feeding cellulytic enzymes on performance, digestibility and ruminal fermentation in goats. Anim. Nutri. Feed Technol. 14:121-136.

Kholif, A.E., Baza-García, L.A., Elghandour, M.M., Salem, A.Z., Barbabosa, A., Dominguez-Vara, I.A. and Sanchez-Torres, J.E. 2016. *In vitro* assessment of fecal inocula from horses fed on high-fiber diets with fibrolytic enzymes addition on gas, methane, and carbon dioxide productions as indicators of hindgut activity. J. Equine Vet. Sci., 39:44-50.

Kholif, A.E., Elghandour, M.M.Y., Rodríguez, G.B., Olafadehan, O.A., Salem, A.Z.M., 2017. Anaerobic ensiling of raw agricultural waste with a fibrolytic enzyme cocktail as a cleaner and sustainable biological product. J. Clean. Prod. 142:2649–2655.

Kozloski ,G.V., Stefanello, C.M., Mesquita, F.R., Alves, T.P., Ribeiro Filho, H.M.N., Almeida, J.G.R. and Moraes Genro, T.C. 2014. Technical note: Evaluation of markers for estimating duodenal digesta flow and ruminal digestibility:

Acid detergent fiber, sulfuric acid detergent lignin, and n-alkanes. J. Dairy Sci. 91:1730–1735.

Krause, D.O., Denman, S.E., Mackie, R.I., Morrison, M., Rae, A.L., Atwwod, G.T. and McSweeney C.S. 2003. Opportunities to improve fiber degradation in the rumen: microbiology, ecology, and genomics. FEMS Microbiol. Rev., 27:663-993.

Kumar, N., Singh, U.B. and Verma, D.N. 1981. Effect of different levels of dietary protein and energy on growth of male buffalo calves. Indian J. Anim. Sci. 51:513-517.

Kumar, U., Sareen, V.K. and Singh, S., 1997. Effect of yeast culture supplement on ruminal microbial populations and metabolism in buffalo calves fed a high roughage diet. J. Sci. Food Agric. 73:231-236.

Kung, L., Treacher, R.J., Nauman, G.A., Smagala, A.M., Endres, K.M. and Cohen, M.A. 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. J. Dairy Sci., 83:115–122.

Lara Bueno, A., Mendoza Martínez, G.D., Hernández García, P.A., Martínez García, J.A. and Plata Pérez, F.X. 2013. Evaluation of high doses of exogenous fibrolytic enzymes in lambs fed an oat straw based ration. Anim. Nutr. Feed Technol., 13:355-362.

Leng, R.A. 1990. Factors affecting the utilization of ‘poor quality’ forages by ruminants particularly under tropical conditions. Nutrition Research Reviews 3:277-303.

Lewis, G.E., Sanchez, W.K., Hunt, C.W., Guy, M.A., Pritchard, G.T., Swanson, B.I. and Treacher R.J., 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. J. Dairy Sci. 82:611-617.

Lila, Z.A., Mohammed, N., Yasui, T., Kurokawa, Y., Kanda, S. and Itabashi, H. 2004. Effects of a twin strain of saccharomyces cerevisiae live cells on mixed ruminal microorganism fermentation *in vitro.* J Anim Sci 82(6):1847-54.

Lin, Y., Vonk, R. J., Slooff, M. J. H., Kuipers, F. and Smit, M. J. 1995. Differences in propionate-induced inhibition of cholesterol and triacylglycerol synthesis between human and rat hepatocytes in primary culture. British J. Nutr. 74:197–207.

López, D., Elghandour, M.M.Y., Salem, A.Z.M., Vázquez-Armijo, J.F., Salazar, M.C. and Gado, H.D. 2013. Influence of exogenous enzymes on *in vitro* gas production kinetics and dry matter degradability of a high concentrate diet. Anim. Nutr. Feed Technol. 13:527-536.

López, S.M.A., Arellano, G.E., Barreras, S.A., González, V.V.M., May, G.D., Plascencia, J.A. y Avery, Z.R. 2006. Influencia de una enzima fibrolítica exógena y el proceso de maceración en un forraje de baja calidad sobre digestión y función ruminal en vacas Holstein secas. Vet Méx. 37(3):275-289.

Madigan, M., Martinko, J.M. and Parker, J. 2004. BROCK Biología de los microorganismos"; 10º ed. Pearson Educación; Madrid.

Mao, H.L., Wu, C.H., Wang, J.K. and Liu, J.X. 2013. Synergistic effect of cellulase and xylanase on *in vitro* rumen fermentation and microbial population with rice straw as substrate. Anim. Nutr. Feed Technol. 13:477-487.

McCallister, A.T., Hristov, A.N., Beauchemin, K.A., Rode, L.M. and Cheng, K.J. 2001. Enzymes in ruminat diets. In: Enzymes in Farm Animal Nutrition. Bedford, M.R., Partridge, G.G. (Eds) CABI Publishing, UK. pp: 145-160.

McCullough H. 1967. The determination of ammonia in whole blood by direct colorimetric method. Clinica Chimica Acta 17, 297–304.

Meale, S. J., Beauchemin, K.A., Hristov, A.N., Chaves, A.V. and Mcallister, T.A., 2014. BOARD-INVITED REVIEW: Opportunities and challenges in using exogenous enzymes to improve ruminant production. J. Anim. Sci. 92:427–442.

Mendoza, G. D., Loera-Corral, O., Plata-Perez, F. X., Hernández-García, P. A., 2014. Considerations on the use of exogenus fibrolityc enzymes to improve forage utilization. Sci. World J. ID 247437 p 9.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Schneider, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. Agr. Sci., 93:217–222.

Merchen, N. R. 1988. Digestión, absorción y excreción en los rumiantes. *En*: El rumiante, fisiología digestiva y nutrición. Church, D. C. (ed.). Acribia, Zaragoza, España. pp:191-223.

Moloney, A. P. and Drennan, M. J. 1994. Effect of yeast culture on growth of beef cattle fed on grass silage plus barley-based concentrates. Ir. J. Agric. Food Res. 32:125.

Montañez, V.O.D., Salinas, C.J. and Salem, A.Z.M. 2013. Utilization fibrolytic enzymes in ruminant feeding. Salem, A.Z.M. (Ed). Nova Science Publishers. New York, USA. pp. 77-93.

Moore, K.J. and Jung, H.G. 2001. Lignin and fiber digestion. J. Range Manage 54:420-430.

Moreno, R., Pinos-Rodríguez, J.M., González, S., Álvarez, G., García, J.C., Mendoza, G. Y Bárcena R. 2007. Efecto de enzimas fibrolíticas exógenas en la degradación ruminal In vitro de dietas para vacas lecheras. Interciencia. 32(12):850-853.

Morgavi, D.P., Beauchemin, K.A., Nsereko, V.L., Rode, L.M., Mcallister, M. and Wang, Y. 2000. A *trichoderma* feed enzyme preparation enhances adhesion of fibrobacter succinogenes to complex substrates but not to pure cellulose. In: 25th Conference of Rumen Function, Chicago, IL, USA, pp. 33.

Morgavi, D.P., Beauchemin, K.A., Nsereko, V.L., Rode, L.M., McAllister, T.A., Iwaasa, A.D., Wang, Y. and Yang, W.Z. 2001. Resistance of feed enzymes to proteolytic inactivation by rumen microorganisms and gastrointestinal proteases. J. Anim. Sci., 79:1621–1630.

Morgavi, D.P., Newbold, C.J., Beever, D.E. and Wallace, R.J. 2000. Stability and stabilization of potencial feed additive enzymes in rumen fluid. Enzyme Microb. Technol. 26:171-177.

Morsy, T.A., Kholif, A.E., Kholif, S.M., Kholif, A.M., Sun, X. and Salem, A.Z.M. 2016. Effects of two enzyme feed additives on digestion and milk production in lactating Egyptian buffaloes. Ann. Anim. Sci. 16:209–222.

Mosoni, P., Chaucheyras-Durand, F., Berat- Maillet, C., Forano, E., 2007. Quantification by real time PCR of cellulolitic bacteria in the rumen of sheeps after supplementation of a forage diet with readily fermentable carbohydrates. Effect of a yeast additive. J. Appl. Microbiol. 103:2676-2685.

Mould, F.L., Kliem, K.E., Morgan, R. and Mauricio, R.M., 2005. *In vitro* microbial inoculum: a review of its function and properties. Anim. Feed Sci. Technol., 123:31-50.

Murugesan, G.R. and Persia, M.E. 2015. Influence of a direct-fed microbial and xylanase enzyme on the dietary energy uptake efficiency and performance of broiler chickens. J. Sci. Food Agri. 95:2521–2527.

Li, M., Heckwolf M., Crowe, J.D., Williams, D.L., Magee, T.D., Kaeppler, S.M., de Leon, N. and Hodge, D.B. 2015. Cell-wall properties contributing to improved deconstruction by alkaline pre-treatment and enzymatic

hydrolysis in diverse maize (*Zea mays* L.) lines. J Exp Bot 66(14):4305–4315.

Newbold, C.J., Rode, L.M., 2006. Dietary additives to control methanogenesis in the rumen. Int. Congr. Ser. 1293:138-147.

Newbold, C.J., Wallace, R.J., McIntosh, F.M., 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. Br. J. Nutr. 76:249-261.

Nkrumah, J.D., Okine, E.K., Mathison, G.W., Schmid, K., Li, C., Basarab, J.A., Price, M.A., Wang, Z., Moore, S.S., 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J. Anim. Sci. 84(1):145-153.

NORMA Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio.

NRC. 1985. Nutrient Requirements of Sheep, 6th Revised edn. National Academy Press, Washington, DC, USA.

NRC. National Research Council. 2001. Nutrient Requirements of Domestic Animals. Nutrient Requirements of Dairy Cattle. 7a ed. National Research Council. National Academy of Sciences. Washington, D. C. USA. pp:381.

NRC. National Research Council. 2007. Nutrient Requirements of Small Ruminants. National Academy of Sciences. Washington, DC.

Nsereko, V.L., Beauchemin, K.A., Morgavi, D.P., Rode, L.M., Furtado, A.F., McAllister, T.A., Iwaasa, A.D., Yang, W.Z., Wang, Y., 2002. Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. Can. J. Microbiol. 48, 14–20.

Oakley, A.J., Lo Bello, M., Nuccetelli, M., Mazzetti, A.P. and Parker, M.W. 1999. The ligandin (non-substrate) binding site of human Pi class glutathione

transferase is located in the electrophile binding site (H-site). J. Molecular Biol. 291:913–926.

Oba, M. and Allen, M.S. 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 3. Digestibility and microbial efficiency. J. Dairy Sci., 83; 1350–1358.

Orpin, C.G. and Joblin, K.N. 1997. The rumen anaerobic fungi. *In*: The Rumen Microbial Ecosystem. Hobson, P.N. and Stewart, C.S., Eds., Blackie, Melbourne. pp. 140-195.

Ørskov, E.R. and Ryle, R. 1990. Energy Nutrition in Ruminants. Elsevier Science Publishers, New York.

Ortíz-Rodea, A., Noriega-Carrillo, A., Salem, A.Z.M., Castelan Ortega, O. y González-Ronquillo, M. 2013. The use exogenous enzymes in dairy cattle on milk production and their chemical composition: A meta-analysis. Anim Nutr Feed Technol. 13:399-409.

Patra, A.K., 2012. The use of live yeast products as microbial feed additives in ruminant nutrition. Asian J. Anim. Vet. Adv. 7:366-375.

Paya, H., Taghizadeh, A., Janmohammadi, H., Moghadam, G.A., 2007. Nutrient digestibility and gas production of some tropical feeds used in ruminant diets estimated by the *in vivo* and *in vitro* gas production techniques. Am. J. Anim. Vet. Sci. 2:108-113.

Pinos-Rodríguez, J.M., González, M.S.S., Mendoza, M.G.D., Bárcena, G.R. y Cobos, P.M. 2002a. Efecto de enzimas fibrolíticas exógenas en la digestibilidad *in vitro* de la pared celular de heno de alfalfa (*Medicago sativa*) o de ballico (*Lolium perenne*). Interciencia. 27(1):28-32.

Pinos-Rodríguez, J.M., González, S.S., Mendoza, G.D., Bárcena, R., Cobos, M.A., Hernández, A. and Ortega M.E. 2002b. Effects of exogenous fibrolytic

enzyme on ruminal fermentation and digestibility of alfalfa and rye-grass hay fed to lambs. J. Anim. Sci. 80:3016-3020.

Pinos-Rodríguez, J.M., Moreno, R., González, S.S., Robinson, P.H., Mendoza, G. and Álvarez, G. 2008. Effects of exogenous fibrolytic enzymes on ruminal fermentation and digestibility of total mixed rations fed to lambs. Anim. Feed Sci. Technol. 142: 210-219.

Polyorach, S., Wanapat, M., Cherdthong, A., 2014. Influence of yeast fermented cassava chip protein (YEFECAP) and roughage to concentrate ratio on ruminal fermentation and microorganisms using *in vitro* gas production technique. Asian Australas. J. Anim. Sci. 27:36–45.

Poutanen, K., Tenkanen, M., Korte, H. and Puls, J. 1991. Accessory enzymes involved in the hydrolysis of xylans. *In*: Enzymes in Bio-mass Conversion. Leatham, G.F., Ed., American Chemical Society, Washington, DC. pp. 426-436.

Ranilla, M.J., Tejido, M.L., Giraldo, L.A., Tricarico, J.M. and Carro, M.D. 2008. Effects of an exogenous fibrolytic enzyme preparation on *in vitro* ruminal a fermentation of three forages and their isolated cell walls. Anim. Feed Sci. Technol. 145:109–121

Reynoso, G.E., Cervantes, R.M., Figuroa, V.J.L., Morales, T.A., Araiza, P.A. and Hernández, J.Y. 2010. Nivel de proteína, fibra, y cultivo de levadura *Saccharomyces cerevisiae* en dietas a base de trigo para cerdos. Agrociencia. 44:753-762.

Robyt, J.F. and Whelan, W. J. 1972. Reducing value methods for maltodextrins: 1. Chain-length dependence of alkaline 3,5-dinitrosalicylate and chain length independence of alkaline copper. Analytical Biochemistry 45:510–516.

Rode LM, Yang WZ, Beauchemin KA. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J Dairy Sci. 82:2121–2126

Rodriguez, M.P., Mariezcurrena, M.D., Mariezcurrena, M.A., Lagunas, B.C., Elghandour, M.M., Kholif, A.M., Kholif, A.E., Almaráz, E.M., Salem, A.Z., 2015. Influence of live cells or cells extract of *Saccharomyces cerevisiae* on *in vitro* gas production of a total mixed ration. Ital. J. Anim. Sci. 14(4):590-595.

Ruiz, P.J.A, Ortíz, R.A., Peñuelas-Rivas, G., Morales. O.A., Gutierrez, M.G., Pescador, P.N. and Gonzálz-Ronquillo, M. 2013. Effect of the addition of enzymes on chemical composition and *in vitro* gas production of hybrid maize varieties preserved by silage in the highlands. Anim. Nutr. Feed Technol. 13:572-582.

Saha, B.C. 2003. Hemicellulose bioconversion. J. Ind. Microbiol. Biotechnol. 30:279-291.

Salem, A.Z.M., El-Adaw, M.M., Gado, H., Camacho, L.M., González-Ronquillo, M., Alsersy, H. and Borhami, B. 2011. Effects of exogenous enzyme on nutrients digestibility and growth performance in sheep and goats. Trop. Subtrop. Agroecosystems. 14:867-874.

Salem, A.Z.M., Hassan, A.A., Khalil, M.S., Gado, H.M., Alsersy, H. and Simbaya, J. 2012. Effects of sun-drying and exogenous enzyme nutrients intake, digestibility and nitrogen utilization in sheep fed Atriplex halimus foliages. Anim. Feed Sci. Technol. 171:128-139.

Salem, A.Z.M., Gado, H.M., Colombatto, D. and Elghandour, M.M.Y. 2013. Effect of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. Livest. Sci. 154:69–73.

Salem, A.Z., Kholif, A.E., Elghandour, M.M., Buendía, G., Mariezcurrena, M.D., Hernandez, S.R. and Camacho, L.M., 2014. Influence of oral administration of Salix babylonica extract on milk production and composition in dairy cows. Ital. J. Anim. Sci., 13(1):10-14.

Salem, A.Z.M., Alsersy, H., Camacho, L.M., El-Adawy, M.M., Elghandour, M.M.Y., Kholif, A.E., Rivero, N., Alonso, M.U. and Zaragoza, A., 2015a. Feed intake, nutrient digestibility, nitrogen utilization, and ruminal fermentation activities in sheep fed *Atriplex halimus* ensiled with three developed enzyme cocktails. Czech J. Anim. Sci. 60(4):185-194.

Salem, A.Z.M., Ammar, H., Kholif, A.E., Elghandour, M.M.Y. and Ortiz, L.B. 2015b. Effect of glucoamylase enzyme extract on *in vitro* gas production and degradability of two diets with 25% of corn or sorghum grains. Indian J. Anim. Sci 85:183–188.

Sales, J. 2011. Effects of *Saccharomyces cerevisiae* supplementation on ruminal parameters, nutrient digestibility and growth in sheep: A meta-analysis. Small Ruminant Research 100:19-29.

SAS, 2002. Statistical Analysis System. User's Guide: Statistics. Ver 9.0. SAS Institute, Cary, NC.

SAS 2006. SAS 9.0 User's Guide: Statistics. SAS Institute, Cary, NC.

Satter, L.D. and Slyter, L.L. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. British J. Nutr. 32:199–208.

SIAP (2015). Servicio de Informacion Agroalimentaria y Pesquera. http://www.siap.gob.mx/cierre-de-la-produccion-agricola-por-cultivo/

Soltan, Y.A., Abdalla, A.L., Silva, L.R.F., Natel, A.S., Morsy, A.S. and Louvandini, H. 2013. Response of different tropical pasture grass species to tratments with fibrolytic enzymes in terms of *in vitro* ruminal nutrient degradation and methanogenesis. Anim. Nutr. Feed Technol. 13:551-568.

Steel, R. G. D., y J. H. Torrie. 1996. Bioestadística: Principios y Procedimientos. Segunda edición. McGraw-Hill. México, D.F. 622 p

Steel, R.G.D., Torrie, J.H. and Dickey, D.A. 1997. Principles and Procedures of Statistics. In: A biometrical approach, 3rd Ed. McGraw Hill Book Co, New York, USA.

Stewart, C.S., Flint, H.J. and Bryant, M.P. 1997. The rumen bacteria. *In:* The Rumen Microbial Ecosystem. Hobson, P.N. and Stewart, C.S. Eds. Blackie, Melbourne. pp. 73-139.

Stock, R.A., Brink, D.R., Britton, R.A., Goedeken, F.K., Sindt, M.H., Kreikemier, K.K., Bauer, M.L. and Smith, K.K. 1987. Feeding combinations of high moisture corn and dry-rolled grain sorghum to finishing steers. J. Anim. Sci. 65:290–302.

Sung, H.G., Kobayashi, Y., Chang, J., Ha, A., Hwang, I.H. and Ha, J.K. 2007. Low ruminal pH reduces dietary fiber digestion via reduced microbial attachment. Asian-Australisian J. Anim. Sci. 20:200-207.

Tajima, K., Aminov, R.I., Nagamine, T., Matsui, H., Nakamura, M. and Benno, Y. 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Applied Environmental Microbiology 67:2766-2774.

Tang, S.X., Zou, Y., Wang, M., Salem, A.Z.M., Odongo, N.E., Zhou, C.S., Han, X.F., Tan, Z.L., Zhang, M., Fu, Y.F., Huang, S.Q., He, Z.X. and Kang, J.H. 2013. Effects of exogenous cellulase source on *in vitro* fermentation characteristics and methane production of crop straws and grasses. Anim. Nutr. Feed Technol. 13:489-505.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B. and France, J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48:185–197.

Tirado-Estrada, G., Mendoza-Martínez, G.D., Pinos-Rodríguez, J.M., Quezada-Tristán, T. and Guevara-Lara, F. 2011. Effects of two fibrolytic enzyme

mixtures on growth performance, digestion and ruminal fermentation in lambs fed corn stover based diets. J. Appl. Anim. Res. 39:158-160.

Togtokhbayar, N., Cerrillo, S.M.A., Jigjidpurev, S., Shinekhuu, J., Urantulkhuur, D., Nergui, D., Elghandour, M.M.Y., Odongo, N.E. and Kholif, A.E. 2015. Effect of exogenous xylanase on rumen *in vitro* gas production and degradability of wheat straw. Anim. Sci. J. 86:765–771.

Treacher, R.J. and Hunt, C.W. 1996. Recent developments in feed enzymes for ruminant rations. In: Proc. Pacific Northwest Animal Nutrition Conference, Seattle, WA, USA, pp. 37–54.

Valdes, K.I., Salem, A.Z.M., López, S., Alonso, M.U., Rivero, N., Elghandour, M.M.Y., Domínguez, I.A., Ronquillo, M.G., and Kholif, A.E., 2015. Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. J. Agr. Sci. 153(04):732-742.

Vallejo, L.H., Salem, A.Z.M., Kholif, A.E., Elghangour, M.M.Y., Fajardo, R.C., Rivero, N., Bastida, A.Z., Mariezcurrena, M.D., 2016. Influence of cellulase or xylanase on the *in vitro* rumen gas production and fermentation of corn stover. Indian J. Anim. Sci. 86(1):70-74.

Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, N.Y. USA p: 374.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74:3583–3597.

Vargas, J.M., Mendoza, G.D., Rubio-Lozano, M.D.S. and Castrejón, F.A. 2013. Effect of exogenous fibrolytic enzimes on the carcass characteristics and performance of grain-finished steers. Anim. Nutr. Feed Technol. 13:435-439.

Vázquez, C.J.C., Estrada, F.J.G., García, W.L.R. and Barbabosa, P.A. 2013. Uses of *Saccharomyces cerevisiae* as feed additive in horse feeding. *In*: Nutritional strategies of animal feed additives. Salem, A.Z.M. (Ed). Nova Science Publishers. New York, USA. pp. 97-104.

Velázquez-Garduño, G., Mariezcurrena-Berasain, M.A., Salem, A.Z., Gutiérrez-Ibañez, A.T., Bernal-Martínez, L.R., Pinzón-Martínez, D.L., Kholif, A.E., Odongo, N.E., Mariezcurrena-Berasain, M.D., 2015. Effect of organic selenium-enriched yeast supplementation in finishing sheep diet on carcasses microbiological contamination and meat physical characteristics. Ital. J. Anim. Sci. 14(3):3836.

Vermerris, W. 201. Survey of genomics approaches to improve bioenergy traits in maize, sorghum and sugarcane. J. Integr Plant Biol. 53:105-119.

Vogel J. 2008. Unique aspects of the grass cell wall. Current Opinion in Plant Biology 11:301–307.

Wang, Y., Mcallister, T.A., Rode, L.M., Beauchemin, K.A., Morgavi, D.P., Nsereko, V.L., Iwaasa, A.D. and Yang, W. 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the Rumen Simulation Technique (Rusitec). British J. Nutr. 85:325–332.

Wang, Y., Spartling, B.M., Zobell, D.R., Wiedmeier, R.D. and McAllister, T.A. 2004. Effect of alkali pre-treatment of wheat straw on the efficacy of exogenous fibrolytic enzymes. J. Anim. Sci., 82:198–208.

Williams, A.G. and Coleman, G.S. 1997. The rumen protozoa. *In*: The Rumen Microbial Ecosystem, Hobson, P.N. and Stewart, C.S., Eds., Blacke, Melbourne. pp. 73-139.

Williams, P.E.V., Tait, C.A.G., Innes, G.M., Newbold, C.J., 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium)

in the diet of cows on milk yield and forage degradation and fermentation patterns in the rumen of sheep and steers. J. Anim. Sci. 69:3016-3026.

Wyman, C. E., Decker, S. R., Himmel, M. E., Brady, J. W., Skopec, C. E., y Viikari, L. 2005. Hydrolysis of cellulose and hemicellulose. *In:* Polysaccharides: structural diversity and functional versatility. Dumitriu, S. Ed. CRC Prees. Quebec, Canada. pp. 1023-1062.

Yang, W. Z., Beauchemin, K. A. and Rode, L. M. 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. Journal of Dairy Science 82:391–403.

Yang, H.J. and Xie, C.Y. 2010. Assessment of fibrolytic activities of 18 commercial enzyme products and their abilities to degrade the cell wall fraction of corn stalks in In vitro enzymatic and ruminal batch cultures. Anim. Feed. Sci. Technol. 159:110-121.

Yescas, Y.R., Bárcena, G.R., Mendoza, M.G.D., González, M.S.S., Cobos, P.M. y Ortega C.M.E. 2004. Digestibilidad *in situ* de dietas con rastrojo de maíz o paja de avena con enzimas fibrolíticas. Agrociencia. 38(1):23-31.