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COLABORACION ACADÉMICA Y DE INVESTIGACIÓN DE CUERPOS ACADÉMICOS

CA-01 NUTRICIÓN ANIMAL

INSTITUTO DE CIENCIA ANIMAL
Carretera Central km 47 ½, A. Postal 24,
San José de las Lajas C.P. 32 700, La Habana, Cuba.
Telef: (53 47) 52 4773 E-mail: ica@ica.co.cu

La Habana, a 11 de junio de 2009
"Año del 50 Aniversario de la Revolución"

Dr. Oscar Ruiz
Universidad Autónoma de Chihuahua
Chihuahua, México

La Dirección de Relaciones Internacionales del Instituto de Ciencia Animal de La Habana, Cuba, se complace en invitarle a visitar nuestro centro, del 22 de Junio al 3 de Julio de 2009.

Su visita permitirá definir nuevas acciones de cooperación como parte del convenio existente entre nuestras instituciones.

Constituirá para nosotros un gran placer recibirle una vez más en nuestras instalaciones y, de este modo, llevar a cabo un programa de trabajo que contribuya a estrechar nuestros lazos de colaboración.

Atentamente,

INSTITUTO DE CIENCIA ANIMAL · CUBA ·

Directora de Relaciones Internacionales
Instituto de Ciencia Animal
La Habana, Cuba

Cuban Journal of Agricultural Science, Volume 47, Number 2, 2013. ICA

Growth dynamics and metabolites produced in the fermentation of *Candida norvegensis* yeast

C. Angulo-Montoya[1], O. Ruiz-Barrera[1], Yamicela Castillo[2], Yoandra Marrero[3], A. Elías[3], C. Arzola[1], P. F. Mancillas[1] and D. Díaz[1]

[1] *Facultad de Zootecnia y Ecología. Universidad Autónoma de Chihuahua, México.*
[2] *Facultad de Medicina Veterinaria, Campus Nuevo. Casas Grandes. Universidad Autónoma de Cd. Juárez, México*
[3] *Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba*
Email: oscaruiz@uach.mx

An experiment was conducted to know the growth dynamics and metabolites produced by a yeast strain with potentialities as activator of the ruminal fermentation. The strain belongs to *Candida norvegensis* and is called Levazoot 15. A completely randomized design, with four repetitions in each study hour, was applied. The variables optic density, biomass, brix degrees, lactic acid and ammoniac nitrogen were determined. The results show that the performance of the optic density and that of the biomass were similar in time. The higher values were reached at 36 h (1.75 and 0.024, respectively). Consequently, the brix degrees diminished from 1.65 at 0 h, up to 0.73 in the 36 h. The levels of ammoniac nitrogen were reduced from 0.70 mM to 0.32 mM in the 12 h. The pH diminished up to the 12 h, but increased slightly from 16 h on. Similar performance was observed in the concentration of lactic acid, diminishing from 3.26 µg to 1.196 µg, for increasing later from 3.244 µg to 36 h. It is concluded that the Levazoot 15 (*Candida norvegensis*) strain showed its maximum growth at 16 h, with pH of 4.17 under the conditions of this study. This is the proper time to use the yeast culture and prove its effect as ruminal activator.

Key words: yeast, dynamic, growth, *Candida norvegensis*.

The feeds production that favors animal production and do not compete with human feeding is one of the main challenges in present cattle rearing. Under these conditions, alternatives to favor the animals' productivity are searched. In the higher part of Mexico, animal feeding is based on pastures and forages of low nutritive value, which do not cover the requirements for maintaining the animals. On this context, the studies are addressed to the use of antibiotics, supplements and additives to improve the ruminal environment and increase the feeding efficiency.

In the case of microbial additives, the yeast *Saccharomyces cerevisiae* and the conidial fungus *Aspergillus orizae*, are within the most beneficial microorganisms for these purposes. They are capable of increasing intake (Erasmus *et al.*1992), the number of cellulolytic bacteria in the rumen (Dawson 1987), the total bacteria and the concentration of short-chain fatty acids (Beharka and Nagaraja 1991). The ruminal pH also increases and the concentration of lactic acid diminishes (Williams *et al.* 1991). On this respect, Wiedmeier *et al.* (1987), Williams *et al.* (1991) and Wohlt *et al.* (1998) demonstrated that the inclusion of preparations with these microorganims increases the ruminal degradability of the ADF and nitrogen (Doreau and Jouany 1998). Besides, milk production increases (Carro *et al.* 1992, Sievert and Shave 1993 and Kung *et al.* 1997) and its chemical composition improves (Yu *et al.* 1997).

It has been proved that strains different from *S. cerevisiae* have potentialities for their use as ruminal activators of high-fiber diets (Lee *et al.* 2000). Castillo (2009) demonstrated in *in vitro* studies that *Candida norvegensis* stimulated the populations of cellulolytic fungi and increased the concentrations of short-chain fatty acids in the DM digestibility of oat straw. For this reason, further studies to increase the knowledge about this yeast are necessary.

The objective of this study was to know the growth dynamics and metabolites produced by the *Candida norvegensis* strain during its fermentation.

Materials and Methods

Localization of the study area. The experiment was conducted in the animal nutrition laboratory of the Husbandry and Ecology Faculty of the Autonomous University of Chihuahua (AUCH), located in the km 1 of the peripheral Francisco R. Almada.

Biological material and culture medium. The strain yeast Levazoot 15 (*Candida norvegensis*) from the collection of the UACH was used, with record number in the Gen Bank: JQ519367.1 GI: 386785959. It was isolated and indentified in previous studies (Castillo 2009) and was kept viable throughout periodic re-culturing in malt extract solution (MES). It was preserved in refrigeration at temperature of 4 °C.

Experimental procedure. For studying the growth dynamics of *Candida norvegensis* yeast, flasks of 250 mL with gauze cap containing 50 mL of the malt extract solution medium were used. They were inoculated with 0.1 % of *C. norvegensis*. The flasks were incubated at 30°C in shaker model I2400, with

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RESEARCH ARTICLE

Feeding of yeast (*Candida* spp.) improves *in vitro* ruminal fermentation of fibrous substrates

Yoandra Marrero[1], Yamicela Castillo[2], Oscar Ruiz[3], Eduviges Burrola[3], Claudio Angulo[3]

[1] *Department of Physiology, Animal Science Institute, San José de las Lajas 32700, Cuba*
[2] *Multidisciplinary Division, Autonomous University of Juárez, Nuevo Casas Grandes 3003, Mexico*
[3] *Faculty of Animal Science and Ecology, Autonomous University of Chihuahua, Chihuahua 31110, Mexico*

Abstract

In vitro gas production technique (IVGPT) was used with the objective of determining the inclusion effect of live cells of two strains of *Candida* yeast on *in vitro* ruminal fermentation of two fibrous substrates. In order to achieve this, two experiments were performed: A) using oat straw (*Avena sativa*) as substrate; B) using alfalfa hay (*Medicabo sativa*) as substrate, comparing the effect of two different strains of *Candida* genre, both isolated from the rumen, on the mentioned substrates. Levica 25 (*Candida tropicalis*) yeast belongs to the culture collection of the Institute of Animal Science, Cuba, and Levazoot 15 (*Candida norvegensis*) yeast is part of the collection of the Faculty of Zootechnology and Ecology of the Autonomous University of Chihuahua, Mexico. Both strains demonstrated their potential in activating the ruminal fermentation. They stimulated (P<0.0001) the ruminal fermentation of the substrates under study. However, the Levazoot strain stimulated the dry matter (DM) fermentation of alfalfa in 21.43%, more than Levica 25. It is concluded that there is an influence of yeast strain and diet on the rumen environment and, therefore, it is important to select the appropriate strain in every production condition.

Keywords: rumen, yeast, gas production, fermentation product, microbial additive

1. Introduction

The use of yeasts in the ruminal production is a good alternative to replace antibiotics as growth promoters (Tripathi and Karim 2011; Durmic *et al.* 2013; Elghandour *et al.* 2014a, b, 2015). Marrero (2011) established, through biochemical and morphological tests, that Levica 25 strain belongs to the *Candida tropicalis* genus and is demonstrated to have an excellent potential to be used as additive in animals consuming fibrous diets compared to a strain of *Saccharomyces cerevisiae* used as control. In the same way, Castillo (2009) showed that Levazoot 15 strain belongs to *Candida norvegensis* and stimulated population of cellulolytic fungus increasing volatile fatty acid (VFA) concentration such as acetic, propionic, butyric, valeric and isovaleric acids and enhancing the *in vitro* dry matter digestibility (IVDMD).

In the world markets, there is an unlimited number of feed additives of microbial origin which use commercial strains of *S. cerevisiae*, in order to produce modifications in the ruminal fermentation patterns (Chaucheyras *et al.* 2008). These products are sold at high prices and most of the times do not produce a desirable effect on animals consuming fibrous diets as those fed in tropical areas. However, there are few

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Yoandra Marrero, Tel: +53-47-599433, E-mail: ymarrero@ica.co.cu

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Identification of *Levica* yeasts as a potential ruminal microbial additive

Y. Marrero[1,2], M.E. Burrola-Barraza[1], Y. Castillo[1], L.C. Basso[3], C.A. Rosa[4], O. Ruiz[1], E. González-Rodríguez[1]

[1]Faculty of Zootechnics and Ecology, Autonomous University of Chihuahua, Chihuahua, Mexico
[2]Institute of Animal Science (ICA), San José de las Lajas, Cuba
[3]Department of Biological Sciences, ESALQ University of Sao Paulo, Sao Paulo, Brazil
[4]Department of Microbiology, Biological Science Institute, Federal University of Minas Gerais, Belo Horizonte, Brazil

ABSTRACT: The objective of this study was to identify and characterize yeast strains isolated from the ruminal ecosystem that are capable of enhancing fermentation in bovines that consume high-fibre diets recommended by livestock feed guidelines in Cuba. The yeasts were isolated from the rumen of Holstein cows that had been fed a biofermented product. Isolated colonies were purified, identified, and characterized using biochemical and molecular methods, and their effects on ruminal fermentation were compared by measuring *in vitro* gas production. Thirteen new strains enhancing gas production with potential use as additives in ruminal fermentation were identified and named *Levica*. These strains grew successfully in detection medium for non-*Saccharomyces* wild yeasts and had long survival periods in the rumen. PFGE analysis found four karyotypes and homology of D1/D2 domain of gene 26S rDNA sequence was similar to that of *I. orientalis*, *R. mucilaginosa*, *P. guilliermondii*, and *C. tropicalis*. Phylogenetic analysis classified the strains into clades A and B. Clade A was further divided into groups AI, AII, BI, and BII. The AI cluster contained *Levica* (*L*)*23*, *L24*, *L29*, *L33*, and formed a monophyletic group with *I. orientalis*, while group AII contained *L18* and formed a monophyletic group with *R. muciloginosa*. The BI cluster contained *L13*, *L15*, *L17*, *L27*, *L28*, and *L32*, all derived from *P. guilliermondii*. Cluster BII was composed only of *L25* located in a separate subclade, forming a monophyletic group with *C. tropicalis*. The most useful strain for preparing microbial feed products to improve ruminal fermentation was *L25* because it showed an increase in gas production.

Keywords: fermentation; bovine; rumen ecosystem

In recent decades interest has grown in the use of yeasts as livestock feed additives. In bovines with high-fibre diets the importance of altering the microbial ecosystem to improve the efficiency of nutrient use has been demonstrated. Around the world one of the most frequent methods of improving ruminal fermentation is the use of biological products called direct-fed microbial feed additives (DFM) obtained from various microorganisms, especially yeasts (Lila et al., 2004; Chaucheyras-Durand et al., 2008).

The addition of these microorganisms to a diet can have a favourable effect on the microbial population and on fermentation indicators in the rumen, thus improving animal health and productivity (Jouany, 2001; El-Ghani, 2004; Stella et al., 2007). The yeast *S. cerevisiae* has been used in some Latin American countries as a supplement in high-fibre diets. Introduction of this yeast has altered fermentation patterns resulting in more efficient utilization and availability of nutrients (Angeles et al., 1995; Mendoza et al., 1995; Doležal et al., 2005), higher cellulolytic bacterial counts (Newbold et al., 1998), higher ruminal and lower lactic acid concentration (Williams et al., 1991), greater digestion of ruminal dry matter (DM),

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In vitro gas production of fibrous substrates with the inclusion of yeast

Yoandra Marrero[1], O. Ruiz[2], A. Corrales[2], O. Jay[4], Juana Galindo[1], Yamicela Castillo[3] and Norma Madera[2]

[1]*Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba*
[2]*Universidad Autónoma de Chihuahua, Periférico Francisco R. Almaáa; km 1 CP 31453. Chihuahua, Chih, México*
[3]*Univeridad Autónoma de Ciudad Juárez, División Multidisciplinaria en Nuevo Casas Grandes de la Universidad Autónoma de Ciudad Juárez. Av. Universidad 3003, Sección Hidalgo. C.P: 31803, Chihuahua, México*
[4]*Centro Universitario de Guantánamo, Carretera a Santiago de Cuba, km 2.5, Guantánamo, Cuba*

The *in vitro* gas production technique was applied for studying the effect of the yeast species and its inclusion dosage on ruminal fermentation of fibrous substrates. Two experiments were executed. In the first, the effect of yeast inclusion of different species on *in vitro* gas production of star grass (*Cynodon nlemfuensis*) was determined and in the second, two levels (5 and 10 mg of DM/mL) of the yeast strain showing the best performance in the first experiment were included for confirming its effect on gas production of maize stubble. Controls without yeast and a blank with the non-inoculated culture were included. As result, all strains had a gas production higher than the control, except in that including strain 15. The best performance was in the one in which strain 27 was incorporated. Strains of *S. cerevisiae* had an intermediate performance regarding the rest. The inclusion of 10 mg DM/mL of the strain 27 (*Pichia guillermondii*) stimulated *in vitro* gas production of the maize stubble until 48 h of fermentation. It is concluded that the species and strain of yeasts, as well as their inclusion dosage, have determinant effect on *in vitro* gas production of different fibrous substrates. This reasserts the importance of selecting the adequate strains for their utilization as additive in diets for ruminants, according to the feed intended to be used.

Key words: *yeasts, Pichia guillermondii, rumen, gas production*

The addition of yeasts as additive in ruminant diets has favorable effects on microbial population and on the ruminal fermentative indicators. Consequently, health and productivity of animals are improved (Stella *et al*. 2007 and Doleža *et al*. 2011). There are multiple products in the world market in which commercial strains of *Saccharomyces cerevisiae* yeast are employed as activators of ruminal fermentation (Chaucheyras *et al*. 2004). However, there are not plenty studies showing the utilization of other yeast genera for these purposes.

Nonetheless, the strain of *Issatchenkia orientalis* dy 252 has been indicated as a potential candidate that could be used as additive (Lee *et al*. 2002). The goodness of yeast genera different from Saccharomyces are known as activators of the ruminal fermentative processes (Galindo *et al*. 2008). In previous studies, Marrero *et al*. (2013) demonstrated that yeast strains of Candida genus species increased *in vitro* gas production of *C. nlemfuensis* when 20 mg DM/mL were included. These backgrounds justify the realization of studies for using yeasts of different species as additive activator of ruminal fermentation. Thus, the objective of this research was to study the effect of the yeast species and its inclusion dosage on *in vitro* gas production of two fibrous substrates.

Materials and Methods

The first experiment was carried out at the laboratory of the Department of Biophysiological Sciences of the Institute of Animal Science, in Cuba and the second at the laboratories of Animal Nutrition of the Faculty of Zootechny of the Autonomous University of Chihuahua, Mexico.

The *in vitro* gas production technique (Theodorou *et al*. 1994) was used. In the first experiment the effect of the inclusion of yeasts of different species on *in vitro* gas production of star grass (*Cynodon nlemfuensis*) was determined. Previous results of Marrero *et al*. (2013) with the same yeast strains were taken into account. In addition, the utilization of a dosage of 10 g DM/animal was assessed, since it is the one generally included in ruminant diets (Rojo *et al*. 2000, Combillas *et al*. 2002 and Beauchemin *et al*. 2003). In the second experiment the *in vitro* gas production of other fibrous substrate (maize stubble) was studied with two levels of yeast strain. This strain had better performance in the first experiment.

Yeast strains. For the study two reference strains of *Saccharomyces cerevisiae* were used: one from the collection of the Cuban Research Institute of Sugar Cane Derivatives (ICIDCA), Cuba and the other isolated from the product LUVUCELL® SC (LLSC). The rest is called LEVICA and belongs to the yeast collection of the Institute of Animal Science, Cuba (RYCASI), with registration number 980 in the World Data Centre for Microorganisms (WDCM). These belong to the species *I. orientalis*, *P. guillermondii*, *R. mucilaginosa*, *C. tropicales* and *Yarrowia lipolitica* and their gene sequences are found in the Gen Bank (Marrero *et al*. 2013).

Reporte de actividades realizadas por el cuerpo académico externo del Instituto de Ciencia Animal (ICA) de la Republica de Cuba por invitación del cuerpo académico de nutrición animal (CA1) de la Facultad de Zootecnia de la Universidad Autónoma de Chihuahua.

Invitados	Actividades realizadas
Dr. Orestes LaO León	➢ Colaboración tecnológica y científica en el proyecto auspiciado por la fundación Produce "Paquete tecnológico para el mejoramiento de esquilmos agroindustriales y su incorporación en raciones para ganado" en el rancho "Los Amanes" propiedad de la I.Z. Carolina Melendez G. ➢ Taller de trabajo con productores de la empresa lechera "ALCODESA" en la ciudad de Delicias, Chihuahua para futuras colaboraciones en esta rama de la producción pecuaria. ➢ Taller de trabajo con productores de ganado de carne de la Unión Ganadera Regional de Chihuahua del grupo "Ganaderos Amigos" en la ciudad de Chihuahua, Chih para futuras colaboraciones en esta rama de la producción pecuaria. ➢ Impartición de plática magistral "Biotecnología de campo: una alternativa para pequeños productores" en el evento "Ciclo de conferencias sobre el papel de la investigación en la educación superior". ➢ Asesorías a estudiantes de posgrado (maestría y doctorado) en labores de tesis y disertaciones del departamento de nutrición animal. ➢ Reuniones científicas con los integrantes del cuerpo académico de nutrición animal para la realización conjunta de investigaciones futuras en las líneas de generación y aplicación del conocimiento que cultivan ambos cuerpos académicos. ➢ Reunión de trabajo con el grupo de investigadores de la Facultad de Zootecnia con el objetivo de estrechar los lazos de investigación en otras áreas

	tales como recursos naturales, reproducción y genética entre otras. ➢ Reunión de trabajo con el grupo de investigadores del Instituto de Ciencias Biomédicas de la Universidad Autónoma de Ciudad Juárez (UACJ) para firmar un convenio de colaboración con el ICA (Cuba).
Dr. Rafael Herrera	➢ Impartición de tres pláticas magistrales "Avances en la investigación en pastos y forrajes", "Vinculación de la investigación con la educación en Cuba" y "Políticas y normativas para la publicación de artículos científicos en la Revista Cubana de Ciencia Agrícola" en el evento "Ciclo de conferencias sobre el papel de la investigación en la educación superior". ➢ Reuniones científicas con los integrantes del cuerpo académico de nutrición animal para la realización conjunta de investigaciones futuras en las líneas de generación y aplicación del conocimiento que cultivan ambos cuerpos académicos. ➢ Reunión de trabajo con el grupo de investigadores de la Facultad de Zootecnia con el objetivo de estrechar los lazos de investigación en otras áreas tales como recursos naturales, reproducción y genética entre otras. ➢ Reunión de trabajo con el grupo de investigadores del Instituto de Ciencias Biomédicas de la Universidad Autónoma de Ciudad Juárez para firmar un convenio de colaboración con el ICA (Cuba).
M.C. Osmany Cardentey León	➢ Entrenamiento y procesamiento de muestras de suero sanguíneo en el laboratorio de nutrición animal de la Facultad de Zootecnia del proyecto nacional de Cuba que ejecuta el cuerpo externo sobre Buffalos de río. ➢ Impartición de plática magistral "Investigaciones sobre Buffalo de río en

	Cuba" en el evento "Ciclo de conferencias sobre el papel de la investigación en la educación superior". ➢ Reuniones científicas con los integrantes del cuerpo académico de nutrición animal para la realización conjunta de investigaciones futuras en las líneas de generación y aplicación del conocimiento que cultivan ambos cuerpos académicos. ➢ Reunión de trabajo con el grupo de investigadores de la Facultad de Zootecnia con el objetivo de estrechar los lazos de investigación en otras áreas tales como recursos naturales, reproducción y genética entre otras. ➢ Reunión de trabajo con el grupo de investigadores del Instituto de Ciencias Biomédicas de la Universidad Autónoma de Ciudad Juárez para firmar un convenio de colaboración con el ICA (Cuba).

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Research Note

Effect of Repeated Suboptimal Chlorate Treatment on Ruminal and Fecal Bacterial Diversity

R. COPADO,[1] C. ARZOLA,[2]* S. V. R. EPPS,[3] F. RODRIGUEZ-ALMEIDA,[2] O. RUIZ,[2] C. RODRIGUEZ-MUELA,[2] YAMICELA CASTILLO CASTILLO,[4] AGUSTIN CORRAL-LUNA,[2] AND J. SALINAS[5]

[1]*Autonomous University of Nuevo Leon, Monterrey, Nuevo Leon 66451, Mexico;* [2]*Autonomous University of Chihuahua, Chihuahua 31203, Mexico;* [3]*Department of Veterinary Integrative Bioscience, Texas A&M University, College Station, Texas 77843, USA;* [4]*Autonomous University of Ciudad Juarez, Chihuahua 32310, Mexico; and* [5]*Autonomous University of Tamaulipas, Ciudad Victoria, Tamaulipas 87000, Mexico*

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ABSTRACT

The minimal effective dose of sodium chlorate as an intervention to reduce the carriage of pathogenic bacteria in food-producing animals has not been clearly established. The effect of low-level oral chlorate administration to ewes was assessed by comparing the diversity of prominent bacterial populations in their gastrointestinal tract. Twelve lactating crossed Pelibuey and Blackbelly-Dorper ewes (average body weight, 65 kg) were randomly assigned (four per treatment) to receive a control treatment (TC; consisting of 3 g of NaCl per animal per day) or one of two chlorate treatments (T3 or T9; consisting of 1.8 or 5.4 g of $NaClO_3$ per animal per day, respectively). Treatments were administered twice daily via oral gavage for 5 days. Ruminal and fecal samples were collected daily, starting 3 days before and ending 6 days after treatment, and were subjected to denaturing gradient gel electrophoresis of the 16S rRNA gene sequence amplified from total population DNA. For ruminal microbes, percent similarity coefficients (SCs) between groups varied from 23.0 to 67.5% and from 39.4 to 43.3% during pretreatment and treatment periods, respectively. During the treatment period, SCs within groups ranged from 39.4 to 90.3%, 43.3 to 86.7%, and 67.5 to 92.4% for TC, T3, and T9, respectively. For fecal microbes, SCs between groups varied from 38.0 to 85.2% and 38.0 to 94.2% during pretreatment and treatment periods, respectively. SCs for fecal populations during treatment were most varied for TC (38.0 to 67.9%), intermediate for T9 (75.6 to 92.0%), and least varied for T3 (80.6 to 90.6%). Heterogeneity within and between groups provided no evidence of an effect of chlorate treatment on ruminal or fecal microbial populations.

Escherichia coli and *Salmonella* spp. are common microorganisms in the gastrointestinal microflora of animals. *E. coli* O157:H7 produces hemorrhagic colitis, which is a potentially lethal disease in humans *(6, 11, 22, 41)*. *E. coli* O157:H7 is the second most common etiological agent of bacterial diarrhea in the western Pacific, second only to *Salmonella (40)*. Several outbreaks in humans have been linked to the consumption of meat and milk. In the United States, a case of poisoning was first detected from contaminated burgers in 1982. Approximately 73,000 people in the United States get sick from this strain annually *(23)*. Ruminants are an important source of contamination for this pathogenic bacterium during slaughter *(28, 31)*. Elder et al. *(16)* have shown that approximately 28% of cattle in the United States are infected with *E. coli* O157:H7. Other zoonotic strains of *E. coli* and *Salmonella* species are also considered key causes of neonatal diarrhea in animals *(19, 26, 27, 39)*. Several pre- and postharvest interventions have been used to control these public health risks, but none are completely infallible *(12, 18, 21, 30)*. Sodium chlorate may be an alternative to control bacteria in animal products because intestinal pathogenic bacteria are capable of reducing nitrate to nitrite intracellularly by a membrane-bound nitrate reductase enzyme *(1, 37)*. Such bacterial nitrate reductase enzymes reduce chlorate to form the cytotoxic product chlorite *(37)*. When *E. coli* and *Salmonella* spp. were exposed to chlorate during in vitro incubation of rumen fluid, populations of *E. coli* O157:H7 and *Salmonella* serovar Typhimurium were reduced significantly *(4)*. In vivo studies have indicated that addition of chlorate intraruminally reduces *E. coli* in fecal content of cattle populations *(5)* because they convert chlorate to chlorite intracellularly. Several other authors have published trials that used chlorate as an intervention strategy to control coliforms in cattle and other domestic ruminants *(10–15, 17, 38)*.

This study evaluated the efficiency of low sodium chlorate doses administered orally as a regulator of populations of total coliforms in ewes, as assessed by changes of rumen and intestinal bacterial populations.

MATERIALS AND METHODS

Location of research. This research trial was carried out at the College of Animal Science and Ecology of the Universidad Autonoma de Chihuahua, in Chihuahua City, Mexico. These facilities are located along the Periférico Francisco R. Almada km 1, with geographic coordinates 28°38′N and 106°04′W (Álvarez

* Author for correspondence. Tel and Fax: +52-614-434-03-03; E-mail: carzola@uach.mx.

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Microbial kinetics, fermentative and chemical characteristics in solid state fermentation of apple bagasse

Y CASTILLO-CASTILLO[1], O RUIZ-BARRERA[2], J SALINAS-CHAVIRA[31], C ANGULO-MONTOYA[4], E BURROLA-BARRAZA[5], M MURILLO-ORTIZ[6], M MONTAÑO-G[7] and A ELÍAS-IGLESIAS[8]

Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, México

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ABSTRACT

The objective of this study was to evaluate the microbial kinetics, fermentative and chemical characteristics during solid state fermentation (SSF) of apple bagasse (AB), which was determined over the course of 4 incubation times; 0, 24, 48, 72 h, in a completely randomized design with 4 repetitions. pH values, lactic acid concentration, numbers of total aerobic bacteria, yeast and lactobacilli, dry matter digestibility and neutral detergent fiber digestibility were determined. True and crude protein, neutral and acid detergent fiber were also measured. Results revealed that pH decreased over the 4 sampling times. The lactic acid concentration increased over time. There was a reduction in numbers of total aerobic bacteria. Numbers of lactobacilli also reduced. Yeast populations (CFU/ml) were stable at 24 h, but decreased thereafter. *In vitro* dry matter digestibility (IVDMD) increased during incubation. *In vitro* neutral detergent fiber digestibility (IVNDFD) similarly increased, with a maximum value observed at 72 h. True protein (TP) increased during fermentation, achieving a high value at 24 h; however, crude protein (CP) showed no change during incubation. Neutral detergent fiber (NDF) content did not change during fermentation however acid detergent fiber (ADF) reduced. It is concluded that the increased content of lactic acid and the accompanying decrease in pH during SSF of AB negatively affected the yeast and total bacteria populations whereas true protein content increased likely because of formation of unicellular protein during the process.

Key words: Apple waste, Bacteria, Chemical composition, Fermentation, Yeast

Currently, biotechnological methods such as solid state fermentation (SSF) of agro-industrial byproducts to enrich protein contents for animal feeding are important because of the low cost and easy operation. Apple bagasse (AB) is the most abundant byproduct produced during juice extraction. This residue is rich in soluble carbohydrates and pectin (Vicente *et al.* 2005), and microorganisms like yeasts (Martorell, 2006) which are low in protein but of high biological value (Rodríguez *et al.* 2008). During SSF the microorganisms in the substrate use nitrogen, carbohydrates, minerals and vitamins for their growth and reproduction. Consequently, the final product is enriched in high quality protein and low fiber contents (Rodríguez *et al.* 2006). For instance, Rodríguez *et al.* (2001) reported an increase in true protein in SSF of sugar cane (*Saccharum officinarum*) and sweet potato (*Ipomea batata*) due to propagation of its epiphyte flora. Nevertheless, the research using AB as substrate for SSF is limited. The present research objective was to determine fermentation and chemical characteristics and assess microbial work during SSF of apple bagasse under microaerobic condition.

Present address: [1]ycastillo75@yahoo.com Departamento de Medicina Veterinaria, División Multidisciplinaria, Universidad Autónoma de Cd. Juárez, Avenida Universidad, Sección Hidalgo, 31333 Nuevo Casas Grandes, Chihuahua, México; [2]oscaruiz@uach.mx Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Km 1 Periferico Fco. R. Almada, C. P. 31031 Chihuahua, Chih., México; [3]jsalinasc@hotmail.com Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Matamoros 8 y 9 Z. Centro. C.P. 87100 Cd. Victoria, Tamps. Mexico; [4]c.angulom@hotmail.com Carr. Intern. Km 3.5 Sur, Culiacán, Sinaloa, México; [5]burrolav@uach.mx Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Km 1 Periferico Fco. R. Almada, C.P. 31031 Chihuahua, Chih., Mexico; [6]muom58@hotmail.com Facultad de Medicina Veterinaria y Zootecnia, Universidad Juárez del Estado de Durango, Carr. Durango-Mezquital C P: 34162 Durango, Dgo., México; [7]mmontano5@yahoo.com Instituto de Investigaciones en Ciencias Veterinarias, UABC. Obregón y Julián Carrillo, Col. Nueva, Mexicali, B.C. 21100 México; [8]aelias@ica.co.cu Departamento de Ciencias Biofisiologicas, Instituto de Ciencia Animal, Ministerio de Educación Superior Carr. Central Km 47.5 Apdo P. 24 Mayabeque, Habana, Cuba.

MATERIALS AND METHODS

Location: The present research was performed in the

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DAVID PUBLISHING

Inclusion Levels of Fermented Apple Bagasse on *in Vitro* Rumen Fermentation of Alfalfa Hay

Yamicela Castillo-Castillo[1], Oscar Ruiz-Barrera[2], Eduviges Burrola-Barraza[2], Claudio Arzola-Alvarez[2], Agustin Corral-Luna[2], Carlos Rodriguez-Muela[2] and Manuel Murillo-Ortiz[3]

1. Department of Veterinary Medicine, Multidisciplinary Division, Autonomous University of Juarez City, Nuevo Casas Grandes, Chihuahua 31803, México

2. College of Animal Science and Ecology, Autonomous University of Chihuahua, Chihuahua 31453, México

3. Faculty of Veterinary Medicine and Animal Science, Juarez University of the State of Durango, Durango 34620, Mexico

Abstract: The aim of this study was to assess the effect of inclusion of fermented apple bagasse (FAB) obtained through solid state fermentation on pH, ammonia nitrogen (N-NH_3), volatile fatty acids (VFA) content, *in vitro* dry matter digestibility (IVDMD), lactic acid and microbial counting of alfalfa hay under *in vitro* rumen environment; four levels of FAB were evaluated (0, 0.25, 0.50 and 0. 75 g/dry matter of FAB) replacing 1.5 g dry matter (DM) of alfalfa hay and incubated at different fermentation times (0, 4, 8, 12 and 24 h) using a complete random design with repeated measures on time. Counts of live yeast colonies (6.08, 6.33, 6.24 and 6.51 CFU/mL expressed as log 10) was higher when FAB was included in the different levels up to the 12 h of fermentation ($P < 0.0001$); lactic acid content also increased as FAB was included in the different levels (10.61, 13.86, 16.84 and 14.57 μg/mL) up to the 12 h of incubation ($P < 0.001$). Nevertheless, the other variables measured as pH, N-NH3, VFA, IVDMD, total bacteria and fungi counts, were not affected by the treatments. It is concluded that substitution of FAB by alfalfa hay in an *in vitro* rumen ecosystem positively modified live yeast colonies and lactic acid concentration, without effect on the other fermentative and microbial parameters of the *in vitro* rumen environment, but considering mixes of FAB and alfalfa hay as a quality ingredient for the feeding of ruminants.

Key words: Apple bagasse, alfalfa, microbial, solid state fermentation.

1. Introduction

The search for alternate food sources to feed livestock, to reduce conventional food imports and to reduce production costs has been of prime importance to researchers in recent years. Apple bagasse is a waste product of the juice extraction industry that provides low protein content [1] and is considered a pollutant to the environment. Its use as a food source for ruminants has been improved through a process of solid state fermentation (SSF), which improves the quality and quantity of protein as well as the digestibility of fiber [2, 3]. Many studies show that foods that undergo SSF are processed successfully by ruminants [4, 5]. Ramos [5] found increases in dry matter (DM) digestibility and improved patterns of rumen fermentation when he supplemented two types of foods produced by SSF in cattle consuming elephant grass (*Pennisetum purpureum*). In the state of Chihuahua, one of the main foods for dairy cattle is alfalfa hay because of its high nutritional value, mainly its high crude protein (CP) content (18%-20%) [6]; however, its market value is too high and out of reach to many low-income producers, therefore cost effective alternatives must be found as partial replacement in the diets of ruminants. The objective of this study was to study the effect of inclusion of fermented apple bagasse (FAB) in SSF in the digestive physiology and microbial quality of fibrous materials, such as, alfalfa hay in an *in vitro* rumen system, as well as its feasibility as an ingredient in feed for ruminants.

Corresponding author: Oscar Ruiz-Barrera, Ph.D., research field: animal nutrition. E-mail: oscarruiz80@gmail.com.

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Effects of *Candida norvegensis* Live Cells on *In vitro* Oat Straw Rumen Fermentation

Oscar Ruiz, Yamicela Castillo[1,*], Claudio Arzola, Eduviges Burrola, Jaime Salinas[2], Agustín Corral, Michael E. Hume[3], Manuel Murillo[4], and Mateo Itza[1]

College of Animal Science and Ecology, Autonomous University of Chihuahua, Chihuahua, Chih. 31000, Mexico

ABSTRACT: This study evaluated the effect of *Candida norvegensis* (*C. norvegensis*) viable yeast culture on *in vitro* ruminal fermentation of oat straw. Ruminal fluid was mixed with buffer solution (1:2) and anaerobically incubated with or without yeast at 39°C for 0, 4, 8, 16, and 24 h. A fully randomized design was used. There was a decrease in lactic acid (quadratic, $p = 0.01$), pH, (quadratic, $p = 0.02$), and yeasts counts (linear, $p<0.01$) across fermentation times. However, *in vitro* dry matter disappearance (IVDMD) and ammonia-N increased across fermentation times (quadratic; $p<0.01$ and $p<0.02$, respectively). Addition of yeast cells caused a decrease in pH values compared over all fermentation times ($p<0.01$), and lactic acid decreased at 12 h ($p = 0.05$). Meanwhile, yeast counts increased ($p = 0.01$) at 12 h. *C. norvegensis* increased ammonia-N at 4, 8, 12, and 24 h ($p<0.01$), and IVDMD of oat straw increased at 8, 12, and 24 h ($p<0.01$) of fermentation. Yeast cells increased acetate ($p<0.01$), propionate ($p<0.03$), and butyrate ($p<0.03$) at 8 h, while valeriate and isovaleriate increased at 8, 12, and 24 h ($p<0.01$). The yeast did not affect cellulolytic bacteria ($p = 0.05$), but cellulolytic fungi increased at 4 and 8 h ($p<0.01$), whereas production of methane decreased ($p<0.01$) at 8 h. It is concluded that addition of *C. norvegensis* to *in vitro* oat straw fermentation increased ruminal fermentation parameters as well as microbial growth with reduction of methane production. Additionally, yeast inoculum also improved IVDMD. (**Key Words:** Rumen, Fermentation, Yeast, Oat Straw, Methane)

INTRODUCTION

Agricultural by-products, such as cereal straw from oats, wheat, and corn, constitute a great potential source of ruminant feed energy. Straws have low nutritional value, because of their low nitrogen and high indigestible fiber content. In recent years, yeast-based additives, primarily *Saccharomyces cerevisiae* (*S. cerevisiae*), have been used to increase rumen feed utilization efficiency (Williams et al., 1991; Miller-Webster et al., 2002; Lila et al., 2004; Doležal et al., 2011; Chaucheyras-Durand et al., 2012). The beneficial effects associated with *S. cerevisiae* in animal studies include a greater dry matter (DM) and neutral detergent fiber digestibility. (Plata et al., 1994), as well as a higher feed utilization and milk production (Moallem et al., 2009). *In vitro* studies have also shown that yeast cultures favourably alter microbial fermentation (Marrero et al., 2013; Ye et al., 2014) and stimulate DM and cellulose digestion (Miller-Webster et al., 2002; Lila et al., 2004; Tang et al., 2008). In the same way, Marrero et al. (2015) showed that inclusion of two strains of yeast (Levazot 15 and Levica 25) in the *in vitro* fermentation of oat straw the accumulated gas production had a twofold increase as a result of yeast effect compared to control. Similar findings were reported by Marrero et al. (2014) when a yeast culture

* Corresponding Author: Yamicela Castillo. Tel: +52-636-110-9227, E-mail: ycastillo75@yahoo.com
[1] Department of Veterinary Medicine, Multidisciplinary Division, Autonomous University of Juarez City, Nuevo Casas Grandes, Chih. 31803, México.
[2] College of Veterinary Medicine and Animal Science, Autonomous University of Tamaulipas, Cd. Victoria, Tamps. 87000, México.
[3] Agricultural Research Service, Southern Plains Research Center, Food and Feed Safety Research Unit, United States Department of Agriculture, College Station, TX 77843, USA.
[4] College of Veterinary Medicine and Animal Science, Juarez University of Durango State, Durango, Dgo. 34000, Mexico.
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Probiotic levels, chemical composition and fermentative characteristics in solid state fermentation of paper sludge for animal feeding

Oscar Ruiz-Barrera[1], Yamicela Castillo-Castillo[2*], Lisie Maite Carrillo-Chan[1], Jaime Salinas-Chavira[3], Claudio Arzola-Alvarez[1], Jesús López-Morones[1], Alberto Grado-Ahuir[1]

[1]Faculty of Zootechnology and Ecology, Autonomous University of Chihuahua, Chihuahua, México
[2]Department of Biomedical Sciences, Autonomous University of Juárez City Campus, Nuevo Casas Grandes, Mexico
[3]Faculty of Veterinary Medicine and Zootechnology, Autonomous University of Tamaulipas, Ciudad Victoria, Mexico
Email: *ycastillo75@yahoo.com

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ABSTRACT

The sludge paper of the industry treated with probiotics in solid state fermentation (SSF) could be used as ingredient in rations for animal feeding. This study assessed the effect of four probiotic (Prozoot15®) levels (PT) on chemical and fermentative characteristics in SSF of the paper sludge (PS) at controlled temperature (30˚C) in laboratory scale. The tested treatments (T) were: T1 (0% PS), T2 (50 g/kg PS), T3 (100 g/kg PS) and T4 (150 g/kg PS), which were fermented at 0, 24, 48 and 72 h, according to a completely randomized design, in a 4 × 4 factorial arrangement with six repetitions per sampling. All treatments included (g/kg DM) 300 molasses, 15 urea, 20 ammonium sulfate, 9 calcium carbonate and 5 of vitamin and mineral premix, plus the PS which was substituted by the PT at 0, 50, 100 and 150 g/kg DM. The results showed a decrease in pH in all treatments at 24 h; however the lowest pH was at 72 h of fermentation. At 72 h of fermentation, the PT addition in T4 increased crude protein, true protein and yeast counts ($P < 0.05$), and decreased pH ($P < 0.05$). In all fermentation time, the PT addition increased ether extract, lactic acid and ammonia nitrogen ($P < 0.05$) and decreased dry matter, ash, NDF and ADF ($P < 0.05$). It was concluded that the addition of 150 g/kg of PT in SSF of paper sludge improves crude and true protein, ether extract, lactic acid, and ammonia. This treatment may have potential use in animal feeding.

Keywords: Fermentation; Paper Sludge; Yeast; Chemical Composition

*Corresponding author.

1. INTRODUCTION

It is known that urban industries pollute the environment by dumping their waste, and causing contamination of soil and water (surface and underground). During the industrial processing of paper in Chihuahua City (Northern Mexico), the solid residue known as sludge of paper is produced; the production of this by-product is about 120 tons per day, which could be used as energy source in the ruminant feeding without generating pollution [1]. Due to the increasing amount of paper sludge generated in this industry, it is necessary to find alternatives to use it. The sludge of paper is characterized by its high structural carbohydrates content, low-protein, and high dry matter that contains inorganic matter [2]; these nutrients may be useful in ruminant diets.

For the potential use of this by-product, other components, like probiotics, are necessary to improve their nutritional value. Probiotics are live micro-organisms cultures, which are administered in adequate quantities, confer beneficial effects to the health of host, and are a natural alternative to improve the animal metabolism [3]. In addition, microbial cultures may improve the nutritional characteristics of by-products when they are treated in solid state fermentation. In this process, the production of organic acids, dry matter digestibility, and protein content are increased; also, there is a decrease in pH and cell wall fractions [4].

Together, the sludge of paper and probiotics could be a part of the diet and used as ingredients in the animal production systems. Also, the sludge of paper and pro-

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Chemical composition, intake, ruminal fermentation, plasma metabolites and hormones in range steers

M. Murillo-Ortiz[a]*, E. Herrera-Torres[a], G. Villarreal-Rodríguez[a] and O. Ruiz-Barrera[b]

[a]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Juárez del Estado de Durango, Durango, México;* [b]*Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Chihuahua, México*

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The objective of this study was to determine and compare, seasonally, across two consecutive years the chemical composition, intake, ruminal fermentation and plasma concentrations of glucose (G), urea-N (UN), non-esterified fatty acids (NEFA) and insulin in grazing steers. Data were analysed as repeated measures within a split plot design. The crude protein (CP), metabolisable protein (MP), neutral detergent fibre (NDF) and metabolisable energy (ME) were different within years and seasons ($P < 0.05$). The values for organic matter intake (OMI), CP intake (CPI) and metabolisable protein intake (MPI) were greater in wet season ($P < 0.01$). The pH values were not affected by years and seasons ($P > 0.05$). Ruminal ammonia (NH_3–N) and volatile fatty acid (VFA) concentrations were lower during dry season than during wet season ($P < 0.05$). The plasma concentrations of G, UN, and insulin were higher in the wet season ($P < 0.05$); however, the NEFA concentrations were higher in the dry season ($P < 0.05$). The variables corresponding to chemical composition, intake, ruminal fermentation and concentrations of metabolites and hormones were affected over time by the years and seasons.

Keywords: intake; ruminal fermentation; plasma metabolites; insulin; range cattle

1. Introduction

Some studies report that, as a result of drastic climate change, animals in the north region of México have periods of 90–100 days of favourable grazing conditions and if the number of days is reduced, the survival of these animals may be in jeopardy (González et al. 2007). The rangelands in semi-arid environments tend to vary greatly in quality and quantity which subsequently affects diet composition and selectivity of grazing cattle. Under long-term drought conditions, the evaluation of nutritive quality of the diet selected by grazing cattle across seasons is essential to design strategic programmes of dietary supplementation. However, these evaluations may be complemented with studies of intake, ruminal fermentation and metabolites and hormones concentrations to more precisely establish dietary supplementation needs. As the rangelands mature, digestibility decreases because of decreased nitrogen and increased fibre and lignin contents (Ramírez et al. 2009). These changes may be accompanied by a decrease in intake, ruminal ammonia and total volatile fatty acid (VFA) concentrations. The blood concentrations of glucose, urea-N (UN), non-esterified fatty acids (NEFA) and insulin also are sensitive to changes in the chemical composition and ruminal fermentation of diet consumed by grazing cattle. In fact, these metabolites and hormones are related with the nutritional status of grazing beef cattle. The concentrations of glucose and UN are sensitive at increased levels of energy and protein in the diet. However, in cattle maintained in a negative energy balance, the insulin is low (Roche et al. 2000). The mechanisms by which glucose, NEFA and insulin may influence the nutritional status of grazing cattle have not been elucidated. Owens et al. (1991) suggested that the evaluation of metabolite and hormone profiles is paramount to our understanding of biochemical mechanisms at work in the grazing animal. Although the nutritive quality of diet of grazing cattle is widely known, remarkably there is very little information about metabolite and hormone concentrations in grazing cattle. This study was conducted to determine and compare, seasonally, across two consecutive years the chemical composition, intake, ruminal fermentation as well as the metabolite and hormone concentrations in grazing cattle on a native rangeland of north Mexico.

2. Materials and methods

2.1. Study area

The study was carried over two consecutive years (2008 and 2009) in a medium-sized shrub-grassland

*Corresponding author. Email: mmorti8@yahoo.com.mx

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Chemical Composition, *In vitro* Gas Production, Ruminal Fermentation and Degradation Patterns of Diets by Grazing Steers in Native Range of North Mexico

M. Murillo*, E. Herrera[1], F. O. Carrete[1], O. Ruiz[2] and J. S. Serrato[3]
Faculty of Veterinary Medicine and Animal Science, Juárez University of the State of Durango,
Carretera Durango-Mezquital, Km. 11.5, CP, 34620, Durango, Mexico

ABSTRACT: The objective of the study was to quantify annual and seasonal differences in the chemical composition, *in vitro* gas production, *in situ* degradability and ruminal fermentation of grazing steers' diets. Diet samples were collected with four esophageal cannulated steers (350±3 kg BW); and four ruminally cannulated heifers (342±1.5 kg BW) were used to study the dry matter degradation and fermentation in rumen. Data were analyzed with repeated measurements split plot design. The crude protein, *in vitro* dry matter digestibility and metabolizable energy were higher during the first year of trial and in the summer ($p<0.01$). The values of calcium, phosphorus, magnesium, zinc and copper were higher in summer ($p<0.05$). The gas produced by the soluble and insoluble fractions, as well as the constant rate of gas production were greater in summer and fall ($p<0.01$). The ammonia nitrogen (NH_3N) and total volatile fatty acids concentrations in rumen, the soluble and degradable fractions, the constant rate of degradation and the effective degradability of DM and NDF were affected by year ($p<0.05$) and season ($p<0.01$). Our study provides new and useful knowledge for the formulation of protein, energetic and mineral supplements that grazing cattle need to improve their productive and reproductive performance. (**Key Words:** Rangelands, Minerals, *In situ* Degradation, *In vitro* Fermentation, *In vivo* Fermentation)

INTRODUCTION

Some studies report that, as a result of drastic climate change, the animals in the northern region of México have periods of 90 to 100 d of favorable grazing conditions and if the number of days is reduced, the survival of these animals may be in jeopardy (González et al., 2007). Under long-term drought conditions, the evaluation of nutritive quality of the diet selected by grazing cattle across seasons is essential to design strategic programs of dietary supplementation. However, these evaluations may be complemented with studies of digestion and ruminal fermentation and minerals content, to more precisely establish dietary supplementation needs. The rangelands in semiarid environments tend to vary greatly in quality and quantity of forages, which subsequently affects diet composition and selectivity of grazing cattle (Obiedat et al., 2002). These changes may be accompanied by decreases in chemical composition, ruminal ammonia (NH_3N) and total volatile fatty acid (VFA) concentrations. The disappearance of digesta from the gastrointestinal tract in ruminants is a function of the competing processes of ruminal digestion and passage of undigested residues (Van Soest, 1994). Studies directed toward evaluation of rates of passage and ruminal degradability in ruminants grazing native rangelands should provide insight into the mechanism controlling voluntary intake in such animals (McCollum et al., 1985). Ruminal degradability has been used often to estimate nutritive quality of different grass species (Murillo et al., 2003; Jancík, 2010). Determination of *in vitro* gas production also provides information on fermentation kinetics of the forage consumed by ruminants. The *in vitro* gas production has been widely used to estimate the nutritive quality of different classes of forages (Njidda, 2010). Grazing beef cattle also require a number of minerals

* Corresponding Author: Manuel Murillo Ortiz. Tel: +52-1-618-8262996, Fax: +52-1-618-8100703, E-mail: muom8@yahoo.com.mx
[1] Faculty of Veterinary Medicine and Animal Science, Juárez University of the State of Durango, México.
[2] Faculty of Animal Science and Ecology, Autonomous University of Chihuahua, Mexico.
[3] Faculty of Agriculture and Animal Science, Juárez University of the State of Durango, México.
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USDA
United States Department of Agriculture
Research, Education and Economics
Agricultural Research Service

November 3, 2015

To whom it may concern:

This letter is to certify that Mr. Jose Luis Guevara, graduate student of the Autonomous University of Cihuahua, spent one week (October 12-17, 2015) at my invitation in an academic stay as part of our collaborative project between the USDA/ARS Southern Plains Agricultural Research Center's Food & Feed Safety Research Unit in College Station and the Universidad Autonoma de Chihuahua. The academic stay was in College Station, Texas. This letter has been provided to Mr. Guevara Dr. Ruiz at his request.

Sincerely;

Robin Anderson, PhD
Project Leader

ars
Southern Plains Agricultural Research Center
Food and Feed Safety Research Unit • 2881 F&B Road
College Station, TX 77845
Voice: 979 260-9317 • FAX: 979 260-9332 • E-mail: Robin.Anderson@ars.usda.gov

USDA

United States Department of Agriculture

Research, Education and Economics
Agricultural Research Service

March 9, 2015

To whom it may concern:

This letter is to certify that that Mr. Jesus Lopez-Morones, Dr. Oscar Ruiz's advisee, was awarded with a period of training at the USDA/ARS Southern Plains Agricultural Research Center's, Food & Feed Safety Research Unit in College Station, Texas from July 13-18, 2014. Training was provided by technicians of our laboratory and by Drs. Robin Anderson and Michael Hume on the following techniques:

1. Bacteriological cultures, measurement of methane emissions and fermentation of poultry litter.
2. Competitive exclusion and related techniques.

This letter has been provided to Dr. Ruiz upon his request. Please do not hesitate to contact me should you have any questions.

Sincerely;

Robin Anderson, PhD
Project Leader

ars

Southern Plains Agricultural Research Center
Food and Feed Safety Research Unit • 2881 F&B Road
College Station, TX 77845
Voice: 979 260-9317 • FAX: 979 260-9332 • E-mail: Robin.Anderson@ars.usda.gov

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Effects of *Candida norvegensis* Live Cells on *In vitro* Oat Straw Rumen Fermentation

Oscar Ruiz, Yamicela Castillo[1,*], Claudio Arzola, Eduviges Burrola, Jaime Salinas[2], Agustín Corral, Michael E. Hume[3], Manuel Murillo[4], and Mateo Itza[1]
College of Animal Science and Ecology, Autonomous University of Chihuahua, Chihuahua, Chih. 31000, Mexico

ABSTRACT: This study evaluated the effect of *Candida norvegensis* (*C. norvegensis*) viable yeast culture on *in vitro* ruminal fermentation of oat straw. Ruminal fluid was mixed with buffer solution (1:2) and anaerobically incubated with or without yeast at 39°C for 0, 4, 8, 16, and 24 h. A fully randomized design was used. There was a decrease in lactic acid (quadratic, $p = 0.01$), pH, (quadratic, $p = 0.02$), and yeasts counts (linear, $p<0.01$) across fermentation times. However, *in vitro* dry matter disappearance (IVDMD) and ammonia-N increased across fermentation times (quadratic; $p<0.01$ and $p<0.02$, respectively). Addition of yeast cells caused a decrease in pH values compared over all fermentation times ($p<0.01$), and lactic acid decreased at 12 h ($p = 0.05$). Meanwhile, yeast counts increased ($p = 0.01$) at 12 h. *C. norvegensis* increased ammonia-N at 4, 8, 12, and 24 h ($p<0.01$), and IVDMD of oat straw increased at 8, 12, and 24 h ($p<0.01$) of fermentation. Yeast cells increased acetate ($p<0.01$), propionate ($p<0.03$), and butyrate ($p<0.03$) at 8 h, while valeriate and isovaleriate increased at 8, 12, and 24 h ($p<0.01$). The yeast did not affect cellulolytic bacteria ($p = 0.05$), but cellulolytic fungi increased at 4 and 8 h ($p<0.01$), whereas production of methane decreased ($p<0.01$) at 8 h. It is concluded that addition of *C. norvegensis* to *in vitro* oat straw fermentation increased ruminal fermentation parameters as well as microbial growth with reduction of methane production. Additionally, yeast inoculum also improved IVDMD. (**Key Words:** Rumen, Fermentation, Yeast, Oat Straw, Methane)

INTRODUCTION

Agricultural by-products, such as cereal straw from oats, wheat, and corn, constitute a great potential source of ruminant feed energy. Straws have low nutritional value, because of their low nitrogen and high indigestible fiber content. In recent years, yeast-based additives, primarily *Saccharomyces cerevisiae* (*S. cerevisiae*), have been used to increase rumen feed utilization efficiency (Williams et al., 1991; Miller-Webster et al., 2002; Lila et al., 2004; Doležal et al., 2011; Chaucheyras-Durand et al., 2012). The beneficial effects associated with *S. cerevisiae* in animal studies include a greater dry matter (DM) and neutral detergent fiber digestibility. (Plata et al., 1994), as well as a higher feed utilization and milk production (Moallem et al., 2009). *In vitro* studies have also shown that yeast cultures favourably alter microbial fermentation (Marrero et al., 2013; Ye et al., 2014) and stimulate DM and cellulose digestion (Miller-Webster et al., 2002; Lila et al., 2004; Tang et al., 2008). In the same way, Marrero et al. (2015) showed that inclusion of two strains of yeast (Levazot 15 and Levica 25) in the *in vitro* fermentation of oat straw the accumulated gas production had a twofold increase as a result of yeast effect compared to control. Similar findings were reported by Marrero et al. (2014) when a yeast culture

* Corresponding Author: Yamicela Castillo. Tel: +52-636-110-9227, E-mail: ycastillo75@yahoo.com
[1] Department of Veterinary Medicine, Multidisciplinary Division, Autonomous University of Juarez City, Nuevo Casas Grandes, Chih. 31803, México.
[2] College of Veterinary Medicine and Animal Science, Autonomous University of Tamaulipas, Cd. Victoria, Tamps. 87000, México.
[3] Agricultural Research Service, Southern Plains Research Center, Food and Feed Safety Research Unit, United States Department of Agriculture, College Station, TX 77843, USA.
[4] College of Veterinary Medicine and Animal Science, Juarez University of Durango State, Durango, Dgo. 34000, Mexico.
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USDA

United States Department of Agriculture

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November 3, 2015

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ARS
Southern Plains Agricultural Research Center
Food and Feed Safety Research Unit • 2881 F&B Road
College Station, TX 77845
Voice: 979 260-9317 • FAX: 979 260-9332 • E-mail: Robin.Anderson@ars.usda.gov

USDA

United States Department of Agriculture

Research, Education and Economics
Agricultural Research Service

6 October 2014

To whom it may concern:

This letter is to certify that Dr. Claudio Arzola and Dr. Oscar Ruiz Barrera, both faculty members of the Autonomous University of Chihuahua, spent one week (July 13-18, 2014) at my invitation in an academic stay as part of our collaborative project between the USDA/ARS Southern Plains Agricultural Research Center's Food & Feed Safety Research Unit in College Station and the Universidad Autonoma de Chihuahua. The academic stay was in College Station, Texas. This letter has been provided to the above mentioned faculty members' programs.

Sincerely;

Robin Anderson

Robin Anderson, PhD
Project Leader

ars

Southern Plains Agricultural Research Center
Food and Feed Safety Research Unit • 2881 F&B Road
College Station, TX 77845
Voice: 979 260-9317 • FAX: 979 260-9332 • E-mail: Robin.Anderson@ars.usda.gov

USDA

United States Department of Agriculture

Research, Education and Economics
Agricultural Research Service

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