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EFECTO DE LA COMBINACIÓN DE UREA Y OPTIGEN® SOBRE LAS CARACTERÍSTICAS DE DIGESTIÓN, COMPORTAMIENTO PRODUCTIVO Y ENERGÍA DE LA DIETA EN RUMIANTES CONSUMIENDO DIETAS CON DIFERENTES PROPORCIONES DE ALMIDÓN:FIBRA ÁCIDO DETERGENTE

TESIS

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PRESENTA

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RESUMEN

Como resultado del alto costo de los granos, la sustitución de granos por co-productos tales como los DDGS en las dietas de finalización es una práctica común. Estos cambios provocan que las dietas contengan una menor cantidad de almidón y una mayor cantidad de fibra. Hipotéticamente, la combinación de urea (U) con urea de liberación lenta (Optigen®) en este tipo de dietas debe promover una mejor sincronía entre las dos fuentes de NNP con el almidón (de alta velocidad de la digestión) y la fibra (de baja velocidad de la digestión) promoviendo una mejor síntesis de proteína microbiana y digestión ruminal con el aumento de la energía digestible de la dieta. En las dietas de crecimiento y finalización, los pocos estudios realizados en este campo, se han centrado en evaluar el efecto de Optigen® en sustitución directa de ingredientes ricos en proteínas (Pinos-Rodríguez et al., 2010; Bourg et al., 2012) más como una estrategia para favorecer la sincronía, y ninguna investigación ha examinado el papel de la relación almidón:fibra (A:F) de las dietas de finalización sobre los efectos de la combinación de ambas fuentes de urea en la digestión y la respuesta productiva en los rumiantes en finalización. Por lo anterior, tres experimentos se realizaron para examinar la combinación de urea y Optigen® en una dieta de finalización que contiene diferentes proporciones A:F (rango de 3 a 6) y sus efectos se analizaron mediante polinomios ortogonales; adicionalmente, se incluyó un tratamiento con un A:F intermedio conteniendo únicamente urea para comparar el efecto de la combinación de urea a una misma proporción de A:F. En el experimento 1, cuatro novillos Holstein canulados (213 ± 4 kg) se utilizaron en un diseño cuadrado Latino 4 × 4 para examinar los efectos de los tratamientos sobre la digestión, mientras que en el experimento 2, 40 corderos (36.65 ± 3 kg) fueron utilizados en una prueba de rendimiento productivo y 60 novillos (372.4±15 kg) se utilizaron en el experimento 3. La combinación de urea no afectó el pH ruminal. La digestión ruminal de la MO, FDA, almidón y el N alimenticio no se afectaron por la combinación de urea. En comparación con el tratamiento con urea ($P<0.05$) y dentro del tratamiento de la combinación de ambos fuentes de urea, el flujo de nitrógeno microbiano (NM) al intestino delgado, la eficiencia microbiana ruminal y

la digestión postruminal de N fueron mayores (efecto cuadrático, $P < 0.01$) para la combinación de urea en la proporción de 4.5. La combinación de urea en la proporción 4.5 incrementó ($P = 0.04$) en 2% el aporte de energía digestible sobre el valor de ED esperado. En el experimento 2, no hubo efectos de la combinación de urea en el consumo de materia seca (CMS). La combinación de urea incrementó ($P \leq 0.04$) la ganancia diaria de peso, eficiencia alimenticia y la retención aparente de energía por unidad de CMS. Independientemente de la proporción A:F, la combinación de urea mejoró la relación observada-esperada de energía dietética así como la retención aparente por unidad de MS consumida, resultando máxima (efecto cuadrático, $P = 0.04$) en la relación 4 A:F. En el experimento 3 la combinación de urea disminuyó el consumo de MS sin afectar la ganancia diaria por lo que aumentó la eficiencia energética de la dieta en 8%. No hubo efectos de la combinación de urea en características de la canal. La combinación de urea y Optigen® dieron lugar a efectos positivos sobre el flujo de NM y la ED de la dieta. El rendimiento y la energética de la dieta también se mejoraron con la combinación, pero al parecer las mejores respuestas se observaron cuando hay una cierta proporción (proporción de ~ 4.5) de almidón: fibra ácido detergente en la dieta.

ABSTRACT

As a result of the cost of grains, the replacement of grains by co-products (i.e. DDGS) in feedlot diets is a common practice. This changes causes that diets contain a lower amount of starch and greater amount of fibre. Hypothetically, combining feed grade urea (U) with slow release urea (Optigen®) in this type of diet should promote better synchrony between starch (high-rate of digestion) and fibre (low-rate of digestion) promoting a better microbial protein synthesis and ruminal digestion increasing the digestible energy of the diet. In growing-finishing diets, the few studies conducted on this field have been focused on evaluating the effect of SRU in direct substitution of high-protein ingredients (Pinos-Rodríguez *et al.,* 2010; Bourg *et al.,* 2012) more than as a strategy to promote synchrony, and no research has examined the role of the starch:fibre ratio of the finishing diets on the effects of the combination of both sources of urea on digestion and growth performance in finishing ruminants. For the latter two experiments were performed to examine the combination of Optigen® and U in a finishing diet containing different starch:acid detergent fibre ratios (range from 3 to 6 S:F) this treatments were analysed with orthogonal polynomials; additionally, a treatment with intermediate S:F ratio with urea (0.80% in ration) as the sole source of non-protein nitrogen was used to compare de effect of urea combination at the same S:F ratio. In experiment 1, four cannulated Holstein steers (213 ± 4 kg) were used in a 4 × 4 Latin square design to examine the treatments effects on digestion, while in experiment 2, 40 lambs (36.65 ± 3 kg) were used in a growth-performance trial and 60 crossbreed steers (372.4±15 kg) were used in experiment 3. Urea combination did not affect ruminal pH. Ruminal digestion of OM, ADF, starch and feed N were not affected by urea combination. Compared to the urea treatment ($p<0.05$) and within the urea combination treatment (quadratic, $p<0.01$), the flow of microbial nitrogen (MN) to the small intestine, ruminal microbial efficiency and postruminal N digestion were greater for the urea combination at a S:F ratio of 4.5. The combination of urea at 4.5 S:F improved (2%, $P=0.04$) the digestible energy (DE) more than expected. In experiment 2, there were no effects of the urea combination or S:F ratio on dry matter intake (DMI). Urea combination increased

(P≤0.04) average daily gain (ADG), gain for feed (G:F) and apparent energy retention per unit DMI. Irrespective of the S:F ratio, the urea combination improved the observed-to-expected dietary ratio and apparent retention per unit DMI being maximal (quadratic effect, P = 0.04) at 4 S:F ratio. In experiment 3, urea combination decreased DMI without effect on average daily gain, thus urea combination increased efficiency of diet energy in 8%. There were no effects of urea combination on carcass characteristics. Combining feed grade urea and Optigen® resulted in positive effects on the MN flow and DE of the diet. The growth performance and dietary energetics also were improved with the combination, but apparently the best responses were observed when there is a certain proportion (ratio ~ 4.0) of starch:acid detergent fibre in the diet.

INTRODUCCIÓN

Uno de los temas de interés en los últimos años en corrales de engorda es la búsqueda de estrategias que optimicen la sincronía a nivel ruminal entre los compuestos nitrogenados y los carbohidratos de la dieta a fin de promover una mejor utilización de los nutrientes y aumento en la eficiencia energética, y por su relevancia actual, como una estrategia para reducir el riesgo de contaminación del medio ambiente (Hristov et al., 2011). La retención de N en el rumen es mediada principalmente por la tasa de degradación de los compuestos N e hidratos de carbono y por la energía disponible para el proceso de la síntesis de proteínas (Tedeshi et al., 2002). Se ha observado que en las dietas altas en grano (proporción de almidón *vs.* fibra ácido detergente mayor de 5 a 1) la urea se puede incluir un 50% más que la cantidad recomendada, obteniéndose efectos positivos sobre el rendimiento de crecimiento o en la utilización de energía de la dieta (Milton et al., 1997; Zinn et al., 2003). Esto último puede explicarse en parte por la posible sincronía de la velocidad de la tasa de degradación ruminal entre la urea grado alimenticio y el almidón. Por otro lado, el uso de productos de urea de liberación lenta ha demostrado beneficios en la sincronía de nutrientes en el ganado alimentado con dietas con alto contenido de forraje (> 10% del FDA) como raciones para ganado lechero y ganado en crecimiento (Inostroza et al., 2010; Alvarez-Almora et al., 2011). Actualmente, como resultado del costo de los granos de maíz, la sustitución de grano de maíz por granos de destilería con solubles (DDGS) en las dietas de engorda intensiva es una práctica común (Klopfenstein et al., 2008). Aunque el valor de la energía de los DDGS es similar al grano de maíz (NRC, 1996, 2007; Estrada-Angulo et al., 2013), los DDGS son más bajos en contenido de almidón (<6%) y más alto en su contenido (> 30%) de fibra digestible (Rosentrater, 2012; Carrasco et al., 2013). Por lo tanto, dependiendo del nivel de reemplazo, la proporción de almidón *vs.* fibra en las dietas de finalización se puede disminuir, por ejemplo, de 5.0 a 3.0. Los pocos estudios que han valorado productos de urea de lenta liberación en dietas de finalización se han centrado en

evaluar estos productos como sustitutos de ingredientes ricos en proteínas (Pinos-2Rodríguez et al., 2010; Bourg et al., 2012; Lascano et al., 2012) más como una estrategia para promover la sincronía, y hasta donde se conoce, ninguna investigación ha examinado el papel que puede tener la relación del almidón y la fibra de las dietas de finalización sobre los efectos de la combinación de ambas fuentes de urea sobre la función digestiva, la energía de la dieta y el rendimiento productivo del ganado en finalización.

HIPÓTESIS

La combinación de urea y Optigen® favorecen la sincronización de nutrimentos a nivel ruminal mejorando la síntesis microbiana y la energía de la dieta resultando en un mejor rendimiento productivo. La relación presente de almidón y fibra en la dieta puede afectar el nivel de respuesta a esta combinación.

OBJETIVO

El propósito fue evaluar, mediante tres experimentos, uno de digestión y dos de comportamiento productivo, el efecto de la combinación de urea y Optigen® en dietas de finalización conteniendo diferentes proporciones de almidón:fibra sobre características de digestión y eficiencia microbiana, comportamiento productivo y energía de la dieta y características de la canal.

REVISIÓN DE LITERATURA

La urea es un compuesto orgánico rico en nitrógeno (44.96% de N) que se utiliza como fuente de nitrógeno no proteico (NNP) para la alimentación de los rumiantes. La urea se descompone en amoníaco en el rumen bajo la acción de la ureasa bacteriana (Satter y Slyter, 1974). Los microorganismos en el rumen son capaces de utilizar el amoníaco resultante para formar aminoácidos, que luego se convierten en proteínas disponibles para el rumiante cuando los microbios pasan a tracto posterior donde son digeridos y absorbidos a nivel intestinal (NRC, 1985). Las razones para preferir el uso de la urea sobre otras fuentes de NNP es que el N de la urea es más barato cuando se considera su concentración, además, su presentación facilita también su almacenaje (McPherson y Witt, 1968). Sin embargo, comparada con fuentes de proteínas verdaderas, la urea se utiliza menos eficientemente (Broderick y Reynal, 2009) debido a que la velocidad a la que la urea es degradada en el rumen es mayor que la velocidad de utilización del amoníaco por las bacterias del rumen, esto favorece la acumulación ruminal de amoníaco y su absorción a nivel portal con su posterior excreción en la orina en forma de urea (Huntington, 1989; Highstreet et al., 2010). En tiempos recientes se ha dado relevancia a la excreción de N por parte de los corrales de engorda ya que esto aumenta el riesgo de contaminación al aire y agua por emisiones de amoníaco (Hristov, 2011). Por lo que el uso racional y óptimo de compuestos de NNP en las dietas de finalización del ganado se hace cada vez más relevante. Una de las acciones es utilizar compuestos de urea modificados para una lenta liberación ruminal y así favorecer una mejor sincronía y una mayor retención de N (Garrett et al., 2005).

Metabolismo Proteico en Rumiantes

Las proteínas proveen los aminoácidos requeridos para el mantenimiento de las funciones vitales como reproducción, crecimiento y lactancia. Los animales no-rumiantes necesitan aminoácidos pre-formados en su dieta, pero los rumiantes pueden utilizar otras fuentes de nitrógeno porque tienen la habilidad especial de sintetizar aminoácidos y de formar proteína desde compuestos nitrogenados no proteicos a través de los microorganismos del rumen. Además, los rumiantes poseen un mecanismo para el ahorro de nitrógeno ya que cuando el contenido de nitrógeno en la dieta es baja, la urea, un producto final del metabolismo de proteína en el cuerpo, puede ser reciclado al rumen a través de la saliva o por retro-difusión portal en cantidades suficientes para llenar sus requerimientos mínimos. En las especies no rumiantes, el total de la urea producida siempre se pierde a través de la orina (Chalupa, 1977).

Fuentes y Tipos de N que Llegan al Rumen

Los aportes de N a rumen son el alimento, la sangre (por retro-difusión) y la saliva. Mientras que los tipos de N que llegan a rumen son proteínas verdaderas constituyentes de los alimentos y los compuestos nitrogenados no proteicos que comprenden principalmente los aminoácidos libres, amidas, ácidos nucleicos, aminas, urea, así como nitratos y nitritos. Algo del nitrógeno atmosférico entra en el rumen durante el consumo y éste puede ser fijado por los microorganismos del rumen como *Metabanobacterium ruminantium*, pero la cantidad es pequeña. En borregos, por ejemplo, es menos de 0.7 g N/día (Li Pun et al., 1975).

Formación de NH3 en Rumen a Través de la Transformación de los Compuestos Nitrogenados

Proteínas. De acuerdo a Owens y Berguen (1983), las proteínas de los alimentos son degradados por los microorganismos del rumen vía aminoácidos para formar

amoniaco y ácidos orgánicos (ácidos grasos con cadenas múltiples). Para llevar a cabo la proteólisis en rumen existe la combinación de una actividad específica microbiana así como un gran número de bacterias involucradas en el proceso. Aun cuando los géneros de *Bacteroides* y *Estreptococos* son las más relevantes en la proteólisis, se ha determinado que los géneros *Selenomonas, Eubacterium, Succinivibrio, Lachnospira, Clostridium* y *Bacillus* también tienen participación en la proteólisis (Hazlewood et al., 1983). La actividad proteolítica parece expresarse constitutivamente (Yokohama y Jhonson, 1988) es decir que la actividad enzimática es independiente del substrato presente. De tal forma que las diferencias en la actividad proteolítica causadas por distintas dietas no son debido a los cambios posibles de inducción/represión de la actividad de la enzima ya que estas diferencias están relacionadas más con el tamaño y la composición de la población microbiana (Wallace y McKain, 1991). Esto promueve que las diferentes tipos de proteínas de los alimentos se degraden a diferentes grados y velocidades en el rumen (Orskov, 1992). Por lo tanto, la cantidad de proteína que se degrada en el rumen depende de la actividad proteolítica microbiana, la estructura de las proteínas (que afecta su solubilidad en el medio ruminal), la accesibilidad del alimento a los microbios, el tiempo de retención ruminal, la solubilidad de la proteína y el pH ruminal (NRC, 1985). El crecimiento de bacterias del rumen es estimulada por péptidos y AA que actúan como factores de multiplicación, lo que afecta en última instancia, la velocidad y el grado de degradación de la proteína en el rumen (Argyle y Baldwin, 1989).

Nitrógeno no proteico. Como resultado de los sistemas de alimentación en confinamiento, en la actualidad el principal componente de N no proteico que llega a rumen es la urea, la cual es suplementada directamente a la dieta. Sin embargo, una gran cantidad de otros compuestos de NNP como son los aminoácidos libres, amidas, nitratos y nitritos llegan al medio ruminal (Nolan y Leng, 1972; Leng y Nolan, 1984). Con respecto a los aminoácidos, éstos son rápidamente hidrolizados mediante deaminasas, aminooxidasas y/o descarboxilasas microbianas, la concentración de AA libres en rumen es baja (~1%) ya que

aquellos que no se degradan pasan al tracto posterior en pocas horas (Van Soest, 1994). Los diferentes aminoácidos se degradan a diferentes velocidades y diferentes grados siendo metionina y lisina los que más escape ruminal tienen (Velle et al., 1997). Debido al alto potencial redox que posee el rumen (-250 a -450 mV, Owens y Goetch, 1988) los compuestos son fácilmente reducidos de tal manera que los nitratos pasan a nitritos y estos son reducidos hasta amoníaco, de igual manera sucede con las amidas libres. La urea es altamente soluble en agua (1 g de urea en 1 mL de H_2O), esta es la causa por lo cual la urea es rápidamente hidrolizada por la ureasa microbiana formando dióxido de carbono y amoniaco (Allison y Prosser, 1991). Schwartz et al. (1964) informaron de la hidrólisis completa de la urea dentro de 30 a 90 min después de la infusión intraruminal, y Caffrey et al. (1967) indican que el 95% del carbonato de urea infundido en el rumen expira como dióxido de carbono dentro de los primeros 30 a 40 minutos después de la infusión. Sin embargo, la tasa de producción de amoníaco es algo dependiente del pH. El pH óptimo para la actividad ureolítica es entre 7.7 y 8.5 y disminuye con mayores niveles de suplementación de urea (Cook, 1976). En resumen, una gran parte de las proteínas de la dieta y de los compuestos que no son proteínas que entran en el rumen son degradados por los microorganismos ruminales a péptidos, aminoácidos y finalmente a NH_3 (Hristov y Jouany, 2005). El destino del NH_3 formado en rumen tiene tres destinos principales: a) formación de proteína microbiana, b) absorción a través de la pared ruminal y reciclaje, y c) pasaje a tracto digestivo posterior (Fig. 1).

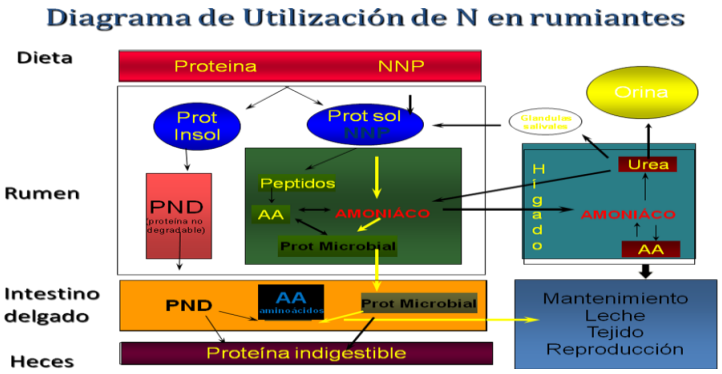

Figura 1. Utilización del nitrógeno en los rumiantes

Formación de Proteína Microbiana.

Si bien es ampliamente reconocido que el amoníaco es el principal nutriente nitrogenado para los microorganismos, se ha estimado que aproximadamente el 80% de las especies presentes pueden crecer con amoníaco como única fuente de N. La ruta para síntesis de proteína microbiana se muestra en la figura 2. Algunas bacterias también pueden obtener desde el 20% hasta el 50% de su proteína de otras fuentes distintas al amoníaco, como péptidos y aminoácidos (Chalupa et al., 1970; Erfle et al., 1977).

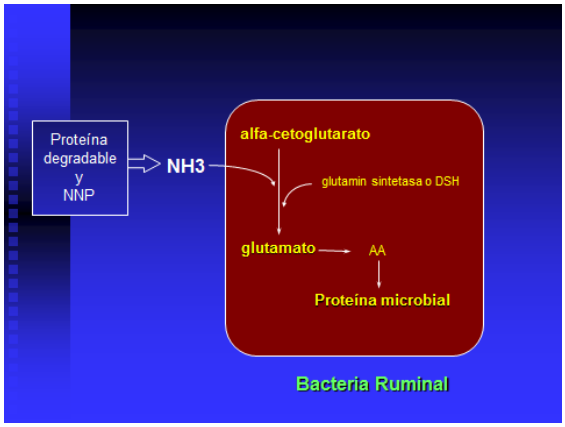

Figura 2. Principal ruta de síntesis de proteína microbiana en rumen (Adaptado de Owens y Zinn, 1988).

Stern y Hoover (1979) en un análisis de la literatura científica disponible en ese momento informaron que se sintetizan aproximadamente 30 g de NM por kg de materia orgánica fermentada (MOF) en rumen, con valores que van de 10 a 50 g de N por kg MOF.

Existen múltiples factores que afectan la síntesis de proteína microbiana en rumen (Phatak, 2008), sin embargo, los 2 factores primordiales son la concentración de NH_3 y la presencia de carbohidratos que aporta la energía y las cadenas carbonadas para la síntesis proteica (Orskov, 1992).

Estudios iniciales in vitro llevados a cabo por Satter y Slyter (1974) indican que la síntesis de proteína microbiana alcanzó el 90% de su máximo con una concentración de 2 mg/dL de amoníaco en el cultivo. Por otra parte, Satter y Roffler (1977) estimaron que la cantidad mínima de amoniaco ruminal necesaria para el apoyo de un crecimiento microbiano máxima es de alrededor de 5 mg /dL, sin embargo, Rogers et al. (1986) mencionan que la concentración de amoniaco en el rumen más adecuado para el crecimiento y la síntesis de proteína microbiana es casi dos veces (9.0 mg/dL) a la concentración estimada por Satter y Roffler (1975). Más recientemente se han indicado, en estudios *in vivo* e *in situ*, valores óptimos entre 17 y 25 mg/dL (Kertz, 2010). Se ha observado una relación entre el contenido de proteína degradable en rumen (PDR) de la dieta y los flujos de N microbial al duodeno (Zinn y Shen, 1998). Estos investigadores indicaron que la síntesis de proteína microbiana se maximiza cuando la PDR era mayor que el 75% de flujo de N microbiano a intestino delgado (equivalente a 100 g de PDR/kg de la MO digestible en tracto total). La cantidad de energía disponible necesaria para una eficiente síntesis de proteína microbiana ha sido extensamente estudiada. En ese sentido, Roffler y Satter (1975) observaron que el amoníaco ruminal fue una función predecible (R^2 = 0.92) del contenido de TDN y del contenido de PC de la dieta: NH_3-N, mg/dL = 38.73 - 3.04PC + 0.171 PC^2 - 0.49 TND +. 0024TND^2. Por otra parte, el NRC (1996) prevé que la eficiencia de la síntesis de proteína microbiana (PCM) tiene una relación con el TDN de alrededor de 13 g de PCM/100g de nutrientes digestibles totales (TDN) consumidos). De igual manera el sistema de potencial de fermentación de urea (UFP) propuesto por Burroughs et al. (1975) el cual considera el nivel de urea que es necesario añadir a las dietas para una eficiencia máxima de síntesis de proteína microbiana y en el que considera el TDN y la cantidad de PDR de la dieta de la siguiente manera:

$$UFP = (.104TND - PDR)/2.8, \text{ o bien:}$$

$$UFP = (3.51NE_m + 1.52 - PDR)/2.8$$

Por ejemplo, si una dieta basal de finalización contiene 2.15 Mcal/kg ENm (87% TDN) y 6% de PDR (es decir, 10% de PC de los cuales el 60% es degradable en rumen), entonces, la cantidad recomendada de urea suplementaria para la dieta basada en su UFP sería de 1.1%. El contenido total de proteína cruda de la dieta (basal más urea) sería así de 13.0%. Sin embargo, en diversos experimentos (Milton et al., 1997; Zinn et al., 2003) se ha demostrado que el sistema de UFP es sobreestimado y esto puede ser debido en gran parte a su incapacidad para tener en cuenta el N reciclado en rumen una vez que es absorbido a través de la pared ruminal.

La proteína microbiana formada en rumen tiene dos destinos potenciales: 1) El reciclaje microbiano por depredación y 2) el paso al tracto digestivo posterior donde es utilizado como fuente proteica por el rumiante (Smith, 1979). La velocidad de flujo de N bacteriano fuera del rumen depende de las concentraciones de bacterias en el líquido y adjunto a las pequeñas partículas que se mueven fuera del rumen. Aunque hay pocas estimaciones satisfactorias de la distribución de microbios en estas dos fases, parece probable que el 75% de las bacterias en el rumen puede estar estrechamente asociado a la porción de la digesta. Las concentraciones de bacterias en las dos fases se ven afectadas por la tasa de crecimiento de las bacterias, la tasa de recambio de la digesta, y la muerte y descomposición de los microbios incluyendo la fagocitosis por protozoos (Cheng y Costerton, 1980).

La biomasa de los protozoos en el rumen varía principalmente como resultado de las características de la dieta. Los protozoos se puede dividir, a grandes rasgos, en ciliados grandes y pequeños, entre los que hay aproximadamente de 50 a 100 veces la diferencia de tamaño. Pocos protozoos se producen en el rumen de los animales en las dietas con alto contenido de grano (probablemente porque estas dietas suelen producir un pH bajo en el rumen, Owens y Goetsch, 1988), pero con el grano restringido en las dietas, éstos podrá superar 5×10^6/mL el fluido del rumen. Los protozoos se han calculado que representan el 40 y el 60% del total de la biomasa microbiana cuando las

concentraciones de los pequeños protozoos superan los 5×10^6/mL o los grandes sobrepasan 5×10^4/mL. Las fuentes de N para el crecimiento de los protozoos son la ingestión de los cloroplastos, la inmersión de las bacterias, y la utilización de partículas de proteína (Weller et al*.,* 1974).

Absorción del NH_3 Formado en Rumen y Reciclaje de N

El amoníaco es absorbido, no sólo en el rumen, sino también de otros las partes del tracto gastrointestinal, como la del omaso, la parte inferior del intestino delgado, y ciego (McDonald, 1988). La absorción de amoníaco se rige tanto por el gradiente de concentración como por el pH. Dado que el amoníaco es una base débil con un pKa de 8.8, el aumento de absorción de amoníaco en un pH mayor es el resultado de un aumento de la concentración de amoníaco, en relación a la concentración de los iones de amonio (NH_4), ya que los primeros pueden penetrar más fácilmente de las capas de lípidos de la mucosa del rumen (Davidovich et al*.,* 1977). Una elevación del pH del rumen se produce durante la alimentación con urea como resultado de la hidrólisis rápida de la urea en dióxido de carbono y amoníaco. Desafortunadamente, la capacidad tampón del líquido ruminal no es tan grande como su capacidad de almacenamiento de ácido (Lana et al., 1998). Así, las condiciones en el rumen favorecen no sólo a la producción rápida de amoniaco, también afectan la tasa de absorción del amoníaco formado. A un pH ruminal de 6.0 sólo 0.16% del amoníaco está en la forma no ionizada. En consecuencia, la toxicidad del amoníaco es mucho menos probable en las dietas de finalización típicas que consumen el ganado de engorda. De hecho, el antídoto para la toxicidad causada por amoniaco es impregnar el rumen con 2.5 a 5.0 litros de ácido acético al 5% (Zinn, 1994).

Reciclaje de Nitrógeno: Cuando hay una falta de energía fermentable o cuando la proteína cruda en la dieta es excesiva, no todo el amoniaco producido en el rumen puede ser convertido a proteína microbiana. Un exceso de amoniaco

pasa la pared del rumen y esta es transportada al hígado. El hígado convierte el amoniaco a urea a través del ciclo de la ornitina la cual es liberada en la sangre. La urea en la sangre puede seguir uno de tres caminos: 1) regresar al rumen vía la saliva y 2) a través de la pared del rumen, o 3) ser excretada por la orina (Owens y Berguen, 1983; Russell et al., 1992). Cuando las raciones son bajas en proteína cruda, la mayoría de la urea está siendo reciclada y poco se pierde en la orina. Sin embargo, mientras más se incrementa la proteína cruda en la ración, menos cantidad de urea es reciclada y más se excreta a través de la orina (Bartley et al*.,* 1976).

La proteína degradable en rumen y el NNP no son la única fuente de N para las bacterias ruminales. Los rumiantes están continuamente reciclando N en el rumen a través de la corriente sanguínea. Esta adaptación permite a los rumiantes sobrevivir consumiendo dietas con muy bajo contenido de N (por ejemplo, menos del 7% de PC).

La cantidad de N reciclado al fluido ruminal disminuye cuando la concentración de NH_3 ruminal es alta o cuando la concentración de N ureico en sangre (BUN) es bajo. Por lo general, el reciclaje del N al fluido ruminal es igual al 10 a 15% de la ingesta dietética de N. Más recientemente, Walpole et al. (2013) informaron que la alta concentración de amoniaco ruminal (15 mg/dL) tendió a inhibir el transporte de urea a través de la pared del rumen cuando la concentración del nitrógeno ureico en sangre (BUN) fue alto (20 mg/dL), sin embargo, no hubo ningún efecto cuando la BUN fue baja (5 mg/dL). La urea entra en el fluido ruminal a través de la saliva y por difusión a través de la pared ruminal. Kennedy y Milligan (1980) observaron que el N reciclado al rumen (NR, expresado como porcentaje de la ingesta de N) era una función predecible (R^2 = 0.97) del nivel de proteína de la dieta (PC, %): NR = 121.7 - 12.01DCP + 0.3235DCP^2.

Suplementación con Fuentes de NNP

Se ha demostrado que los rumiantes pueden convertir el nitrógeno no proteico (NNP) en proteína, las fuentes alternas de NNP son una fuente de reemplazo de proteína atractiva por su bajo costo comparado con la mayoría de las proteínas naturales (Currier et al., 2004). Existen varias fuentes de NNP que han sido probados en las dietas de finalización y a continuación se mencionan algunas de ellas

Ureas. La urea es el nombre común para carbonildiamida (H_2NCONH_2), es un compuesto químico cristalino e incoloro que generalmente se presenta en forma granular, su peso molecular es de 60.06 y su densidad de 1.34 g/cm^3. En su pureza contiene 46.65% de N (Fig. 3), aunque la urea de grado alimenticio contiene típicamente 42 a 45% de N (equivalente a 262 a 281% de PC). A finales del siglo XIX investigadores alemanes (Ehrenberg et al., 1891; Zuntz, 1891) determinaron que la urea podría ser utilizada para reemplazar una porción de la proteína en las raciones de los rumiantes. 50 años más tarde aún no se reconoce ampliamente que la urea se convierte a las proteínas en cantidades importantes

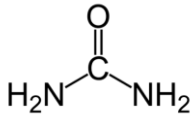

Figura 3. Fórmula de la molécula de urea

en rumiantes (Kertz, 2010). Reid (1953) llegó a la conclusión de su extensa revisión de la literatura que la conversión de urea a las proteínas es mediada por los microorganismos del rumen y retículo, la cual es utilizada por el animal en tracto posterior. Que el NNP puede ser utilizado para sustituir la proteína animal en las dietas de bajo contenido de nitrógeno para rumiantes. Sin embargo, el NNP no suele ser utilizado en las mismas cantidades como proteína natural. Los mismos autores también encontraron que la urea era menos eficaz en las dietas

que contienen 12% o más de PC y que la urea puede reducir la ingesta de alimento cuando la inclusión dietética supera el 1% de la MS. La urea tiene un sabor fresco y salino. Su aceptación o gustosidad en el ganado es alta. Esto debe considerarse en la elaboración de suplementos con urea en los cuales debe evitarse que la urea forme grumos grandes. Grumos de urea de 50 a 100 g pueden ser tóxicos si se consume por terneros ligeros durante el período de recepción o en crecimiento. Se ha demostrado que la ingesta total de alimento suele ser similar para dietas que contiene urea comparada con dietas que contienen proteínas naturales. Sin embargo, la cantidad consumida por vez puede disminuir con la administración de suplementos de urea (Conrad, 1977). Diversos investigadores han cuestionado las recomendaciones originales de Reid cuando se aplican a dietas de finalización (Zinn et al., 1994, 2003; Milton et al., 1997). Los factores que afectan el uso eficiente de urea en dietas de finalización para bovinos de engorda no están bien definidos. Sustentado en informes iniciales sobre el tema, la principal recomendación para el uso de NNP para dietas de finalización es que éste no debe proveer más de un tercio del total de N de la ración, o bien, debe utilizarse, en base seca, un máximo de 1% del total de la dieta (Reid, 1953; Chalupa, 1968; Davidovich et al., 1977; Varady et al., 1979). Sin embargo, no existen fundamentos empíricos o económicos para sustentar esa aseveración. Burroughs (1975) indica que la cantidad máxima de inclusión de NNP puede ser estimada en base al potencial de fermentación de urea el cual se deriva a partir de la síntesis de proteína microbiana y la cantidad de proteína del alimento degradada a nivel ruminal (PDR). Actualmente el NRC (1996; Nivel 1) indica que la cantidad de urea que debe ser añadida a la dieta para optimizar el crecimiento microbiano es equivalente a la síntesis neta de proteína microbiana menos el contenido de la proteína degradable en rumen de la dieta dividido entre 2.8. Ahora bien, en algunos estudios realizados en ganado que consumieron dietas de finalización se han observado beneficios en la digestión de nutrimentos y el comportamiento de crecimiento con la adición de urea por encima de los requerimientos teóricos para optimizar la síntesis de proteína microbiana (Milton et al., 1997; Zinn et al., 2003). Lo anterior puede deberse, por un lado, a que en

esos estudios se dio una posible sincronía óptima entre la liberación de amoniaco y la energía disponible para su retención, y por otro, como resultado de su estructura molecular, al efecto alcalinizante potencial de la urea que favorece un mejor medio para el funcionamiento de la microbiota ruminal (Zinn, 2003). El efecto amortiguador de la urea parece ser sólo en un corto tiempo (1-h) post-consumo ya que en la gran mayoría de los informes (Zinn et al., 1994; Milton et al., 1997; Brake et al., 2010) la suplementación de urea en dietas formuladas a base de maíz, no tiene efecto sobre el pH ruminal. Estos investigadores midieron el pH ruminal en el momento de la alimentación y en intervalos de 2 h (Brake et al., 2010), de 3 h (Milton et al., 1997) o de 4 h después de la alimentación (Zinn et al., 1994). La correcta sincronización de la tasa de degradación de urea con los carbohidratos de la dieta puede resultar en incrementos en la digestión de la MO y de la FDN. Esto podría explicar en parte el por qué en algunos estudios (Milton et al., 1997, Koening y Beauchemin, 2013) la inclusión de urea no afectó la digestión de la MO en tracto total, pero en otros (Zinn et al., 2003, Brake et al., 2010) la inclusión de urea aumentó la digestión MO. Los aumentos en la digestión ruminal del almidón en respuesta a la suplementación de urea se han observado en las dietas de finalización formuladas en base de maíz hojuelado (Zinn et al., 1994) y maíz quebrado (Milton et al., 1997), y en dietas que contienen cebada en hojuelas (Zinn et al., 2003). Esto aunado a respuestas positivas en la digestión ruminal del N y FDN trae como resultado incrementos en la digestión ruminal de la MO (Milton et al., 1997; Bourg et al., 2012) con el consecuente incremento en la síntesis de proteína microbiana (Zinn y Shen, 1998).

Se han observado incrementos en la ganancia o en la eficiencia alimenticia cuando el ganado consume dietas suplementadas con urea. Lo que permanece en controversia es cuál es el nivel adecuado de suplementación para una mejor respuesta. En ese sentido, Tedeshi et al. (2002) reportaron incrementos del 17% sobre la ganancia diaria y de 13% en la eficiencia alimenticia cuando suplementaron con 0.8% de urea en la dieta de finalización. Mientras que, Taylor-Edwards et al. (2009b) informaron que la suplementación con 0.4% de urea a la

dieta aumentó la ganancia de peso y la eficiencia alimenticia, pero el incrementar más el nivel (0.8, 1.2 y 1.6%) no aumentó más la ganancia de peso o la eficiencia. Zinn et al. (1994) observaron un incremento (componente linear, $P<0.05$) del coeficiente de observado-esperado en la EN de la dieta cuando las dietas de finalización fueron suplementadas con 0.0, 0.8 y 1.20% de urea.

Generalmente el suplementar con urea no afecta las características de la canal cuando se compara con dietas suplementadas con proteínas naturales (Tedeshi et al., 2002; Zinn et al., 2003; Taylor-Edwards et al., 2009b). Milton et al. (1997) reportaron respuestas cuadráticas en peso de la canal y área del ojo de la costilla siendo máxima a nivel suplementario de 0.7% y el mínimo en el nivel de urea de 1.40%. Ellos atribuyeron la disminución del peso de la canal a la disminución de la ingestión de MS observada para los novillos que consumieron el nivel de 1.40% de urea.

***Ureas modificadas*.** Los primeros productos de urea de liberación lenta incluyen: biuret, un compuesto estudiado ampliamente que se forma por la condensación de dos moléculas de urea. El “biuret” se ha estudiado en las dietas de rumiantes desde la década de 1970 (Fonnesbeck et al., 1975). El biuret contiene un 41% de nitrógeno, tiene un nivel de gustosidad aceptable, no es tóxico, tiene una lenta liberación de amoníaco en el rumen y una baja solubilidad en agua. La “estarea”, el cual es un producto generado mediante la cocción conjunta de granos y urea para formar un producto que se degrada más lentamente ya que la urea se encapsula en almidón gelatinizado (Deyoe et al., 1968). Otra forma de urea modificada es la que se obtiene mediante la reacción de urea con fosfato para la formación de fosfato di-amónico (Oltjen et al., 1968). Se trata de un polvo cristalino de color blanco soluble en agua. Contiene 21.4% de nitrógeno y 23.7% de fósforo. Tiene la ventaja, con respecto a la urea, que mejora a la vez el aporte de fósforo. Mientras que el polifosfato amónico (PFA) es una fuente común de fósforo y de NNP en los suplementos líquidos. Se emplea en forma líquida, ya que tiene la ventaja, que no es corrosivo. Contiene 11% de nitrógeno y 16.1% de fósforo.

Posteriormente, los esfuerzos para ralentizar la hidrólisis de urea en el rumen se ha logrado mediante la unión de urea a la lignina (Castro et al., 1999) o al cloruro de calcio (Huntington et al., 2006), o bien, mediante la encapsulación de las partículas de urea con polímeros (Galo et al., 2003) o con lípidos (Owens et al., 1980) para reducir la velocidad de liberación en el rumen.

El Optigen® es una fuente de nitrógeno no proteico de liberación controlada. Contiene 41% de N lo cual es equivalente al 256% PC. Consiste en una urea cubierta por un polímero en base de aceite vegetal que libera lentamente el amoníaco a velocidad similar a algunas proteínas de los alimentos (Fig. 4). Esta característica es deseable principalmente en las dietas que requieren un alto porcentaje de proteína, ya que permite incluir una mayor cantidad de urea en la dieta, en forma de Optigen®, teniendo un riesgo reducido de toxicidad. Debido a su reciente introducción al mercado, este producto ha sido evaluado en distintos experimentos.

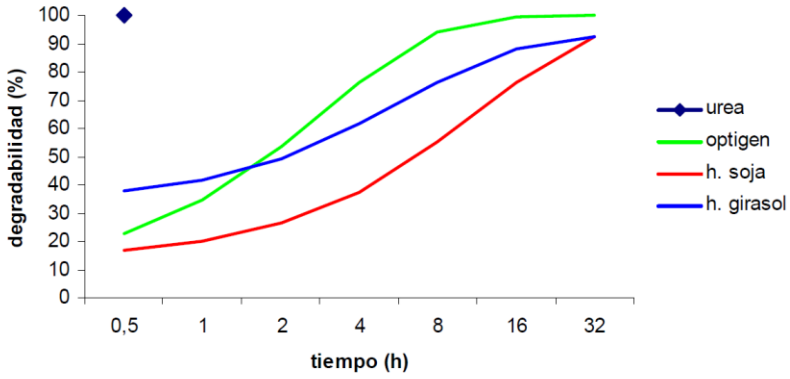

Figura 4. Cinética de degradación ruminal de cuatro fuentes de nitrógeno (INRA, 2002).

En relación a las ureas modificadas (protegidas) mediante polímeros a base de aceites (Optigen ® II, Alltech) se debe considerar que las dietas altas en concentrado promueven a una depresión en el pH del rumen el cual afecta a la

actividad lipolítica ruminal. Se ha observado que la lipólisis ruminal se deprime significativamente a pH por debajo de 6 (Van Nevel y Demeyer, 1996). La degradación enzimática de la capa lipídica de gránulos Optigen ® II es el mecanismo propuesto por el cual se libera la urea. Por lo tanto, la reducción de la lipólisis en el rumen de los animales alimentados con una dieta alta en concentrado puede tener lugar a una liberación más lenta de urea a partir de gránulos Optigen ® II.

Respuestas al Uso de Urea de Liberación Lenta

Dado su bajo costo por unidad de N se han hecho diversos esfuerzos por reducir la toxicidad de la urea para aumentar su uso. Una forma de reducir el amoníaco que llega al hígado es mejorar la eficiencia en la utilización del amoniaco formado en rumen, o bien, modificar su velocidad de tasa de formación (Taylor-Edwards et al., 2009b). Para poder llegar a esto, algunos investigadores han usado inhibidores de ureasa con resultados inconsistentes. Otra forma ha sido el uso de fuentes de N de liberación lenta (ULL). En los últimos años se han logrado regular la liberación al utilizar cubiertas a base de aceites o polímeros de aceites. Las ULL se han formulado originalmente con el objetivo de reducir la toxicidad e incrementar la aceptación de suplementos que contienen urea (Owens et al., 1980).

Golombeski (2006) comparó Ruma Pro®, que es urea recubierta por cloruro de calcio, que permite una liberación lenta. Se comparó la producción lechera de 12 vacas raza Pardo suizo (ocho vacas multíparas y 4 vacas primíparas) en Dakota del Sur usando urea común y Ruma Pro, y distintos niveles de carbohidratos solubles. Las dietas tipo integral contuvieron 16.6% de PC, 30% de FDN y 1.55 Mcal/kg de EN_L y se formularon a base de 50% de forraje. El experimento tuvo una duración de 4 periodos con 28 días de duración por cada periodo. Las vacas se ordeñaron 3 veces al día y se monitorearon para mastitis en

forma semanal. No se observó diferencia estadística en ninguna variable con respecto a la fuente de nitrógeno (Cuadro 1).

Cuadro 1. Consumo, producción y composición de la leche en vacas suplementadas con urea convencional y urea de lenta liberación.

	UC*		ULL*	
Variable	AF**	ANF**	AF	ANF
Consumo de materia seca, kg/d	21.30	21.30	19.70	20.00
Producción de leche, kg/d	26.70	25.50	26.80	25.60
Grasa en la leche, %	4.18	4.51	4.36	4.48
Proteína en la leche, %	3.73	3.75	3.75	3.74

* UC= Urea común, ULL= Urea de lenta liberación.
** AF= Dieta con azúcares fermentables, NAF=dieta sin azúcares fermentables

(Golombeski et al., 2006)

Taylor-Edwards et al. (2009a) también evaluaron otra fuente de urea de lenta liberación (Optigen®) comparando urea y Optigen® a niveles de 0, 0.4, 0.8 y 1.2% de la ración en BM seca la otra dieta contuvo pasta de soya como fuente de proteína natural; las dietas contuvieron 11% de PC en promedio. Los novillos utilizados fueron 180 Angus de 330 kg de peso inicial. La dietas se formularon con base a ensilaje de maíz (90%) y los niveles de proteína fueron de 9.0, 9.9, 10.7, 11.5 y 12.3%. No se pudieron observar ventajas claras al sustituir la urea por ULL, además, esta sustitución podría resultar negativa en el comportamiento productivo del bovino tanto en situaciones de deficiencia de N (0.4%) como en dietas con altas concentraciones de N (1.6%). Sin embargo, una suplementación intermedia (0.8 y 1.2%) afecta de forma similar que la urea en comportamiento productivo del animal (Cuadro 2). Esto coincidió con los resultados de Taylor-Edwards et al. (2009b), que observaron que el comportamiento productivo de los bovinos era similar, pero la concentración de amoniaco en la sangre si se afectaba resultando un pico marcado en el caso de la urea, pero no así en el caso de la ULL. Aun cuando hubo un aumento de amoniaco en el caso de la urea, este incremento no se consideró tóxico porque no estuvo cerca de los valores considerados como críticos.

Cuadro 2. Efecto de la suplementación de urea convencional y de lenta liberación en dietas a base de maíz con becerros en crecimiento.

Variable	GDP, kg/d	Consumo MS, kg/d	Ganancia:Consumo, g:kg
Control			
0	0.85	6.87	123
Urea			
0.4	1.14	7.54	150
0.8	1.16	7.64	152
1.2	1.16	7.58	153
1.6	1.18	7.65	155
Optigen®			
0.4	1.00	7.20	139
0.8	1.18	7.53	157
1.2	1.20	7.60	158
1.6	1.03	7.49	137

(Taylor-Edwards et al., 2009)

Galo et al. *(*2003) evaluaron Optigen® comparándolo contra harina de soya. Se hicieron tres dietas con las dos fuentes distintas de N con la participación de Optigen + urea en dietas con 16 y 18% de PC. La cantidad de Optigen® añadido fue de 0.77% y se combinó con 0.13 y 0.09% de urea. No hubo efecto de los tratamientos sobre el consumo de MS. Se observó que la inclusión de Optigen® no mejoró la producción de los animales en el experimento, y la sustitución parcial de fuente de N de Optigen® por harina de soya redujo (4%) la producción de leche (Cuadro 3).

Cuadro 3. Producción y composición de la leche en vacas suplementadas con diferentes fuentes de nitrógeno y cantidades de urea de liberación controlada.

Variable	CP18 0CU*	CP18+CU*	CP16+CU*
Consumo de materia seca, kg/d	23.6	23.6	23.1
Producción de leche, kg/d	35.6	34.8	33.8
Grasa en la leche, %	3.8	3.6	3.8

*CP18 OCU: dieta con 18%PC sin Optigen; CP18+CU: dieta con 18% con Optigen; CP16+CU: dieta con 16%PC con Optigen.

Tedeschi et al. (2002) evaluaron el Optigen® 1200 en 100 novillos Angus y compararon el desempeño de cuatro lotes con cuatro tratamientos difiriendo en la fuente de N: 100% urea, 100% Optigen, 66:34 Urea:Optigen (U66O34) y 66:34 Optigen:Urea (O66:U34). Observaron que hubo una diferencia estadística entre el tratamiento O100 y U100 en la GDP, siendo U100 superior por 16% con respecto al O100. Sin embargo, también observaron que los tratamientos U66O34 y O66U34 permitieron GDP similares a las U100. Al igual que en GDP, al combinar las fuentes de N, se obtuvo un peso similar al de U100. (Cuadro 4).

Cuadro 4. Efecto de la combinación de urea convencional y urea de lenta liberación (Optigen) sobre comportamiento productivo en becerros.

	Tratamientos*			
Variable	U100O0	U66O34	U34O66	U0O100
Peso Inicial, kg	340	334	335	333
Peso final, kg	542[a]	533[ab]	527[ab]	520[b]
Ganancia diaria de peso, g/d	1.651[a]	1.520[ab]	1.512[ab]	1.419[b]
Consumo de materia seca, kg/d	9.27	9.64	9.06	9.35

*Los tratamientos son cuatro proporciones de urea (U) y Optigen® 1200 (O) (100:0, 66:34, 34:66 y 0:100) (Tedeschi et al., 2002)

Bourg et al. (2012) observaron una tendencia a una mayor ganancia en los novillos que consumieron Optigen® con aquellos novillos que consumieron una dieta isonitrogenada de urea. En contraste, Taylor-Edwards et al. (2009a) informaron que la urea tiende a resultar en mayores ganancias de peso que la urea de liberación lenta, pero también observaron una interacción con la cantidad de urea en la dieta, donde la urea dio lugar a un mejor crecimiento en niveles bajos de suplementación mientras que la urea de liberación lenta fue mejor en los niveles superiores de la suplementación.

CONCLUSIONES

De acuerdo con la literatura revisada, los nuevos productos de urea de lenta degradación ruminal tienen ventajas importantes sobre la urea convencional. Hasta ahora el mayor interés de estos productos es que ofrecen una alternativa útil para reducir la inclusión de concentrados de proteína vegetal en las dietas de los rumiantes, pero la posible contribución a la sincronía ruminal de la energía y el nitrógeno no ha sido convenientemente estudiada.

Por lo anterior, se requieren más investigaciones para establecer las mejores condiciones de utilización en el diseño y formulación de las dietas de finalización que pretendan utilizar estos productos.

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EXPERIMENTO I

Heading title: Slow-release urea and feed grade urea on digestion

Effects of combining feed grade urea and a slow-release urea product on characteristics of digestion, microbial protein synthesis and digestible energy in steers fed diets with different starch:ADF ratios

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ABSTRACT: As a result of the cost of grains, the replacement of grains by co-products (i.e. DDGS) in feedlot diets is a common practice. This changes causes that diets contain a lower amount of starch and greater amount of fibre. Hypothetically, combining feed grade urea (U) with slow release urea (Optigen) in this type of diet should promote better synchrony between starch (high-rate of digestion) and fibre (low-rate of digestion) promoting a better microbial protein synthesis and ruminal digestion with increasing the digestible energy of the diet. For the latter, four cannulated Holstein steers (213 ± 4 kg) were used in a 4 × 4 Latin square design to examine the combination of Optigen and U in a finishing diet containing different starch:acid detergent fibre ratios (S:F) on the characteristics of digestive function. For that, three S:F ratios (3.0, 4.5 and 6.0) were tested using a combination of U (0.80%) and Optigen (1.0%). Additionally, a treatment of 4.5 S:F ratio with urea (0.80% in ration) as the sole source of non-protein nitrogen was used to compare de effect of urea combination at same S:F ratio. The S:F ratio of the diet was manipulated by replacing the corn grain by dried distillers grain with solubles and roughage. Urea combination did not affect ruminal pH. The S:F ratio did not affect ruminal pH at 0 and 2 h post-feeding but, at 4 and 6 h, the ruminal pH decreased as the S:F ratio increased (linear, $p<0.05$). Ruminal digestion of OM, starch and feed N were not affected by urea combination or S:F ratio. The urea combination did not affect ADF ruminal digestion. ADF ruminal digestion decreased linearly ($p=0.02$) as the S:F ratio increased. Compared to the urea treatment ($p<0.05$) and within the urea combination treatment (quadratic, $p<0.01$), the flow of microbial nitrogen (MN) to the small intestine and ruminal microbial efficiency were greater for the urea combination at a S:F ratio of 4.5. Irrespective of the S:F ratio, the urea combination improved (2.8%, $p=0.02$) postruminal N digestion. As S:F ratio increased, OM digestion increased, but ADF total tract digestion decreased.

The combination of urea at 4.5 S:F improved (2%, P=0.04) the digestible energy (DE) more than expected. Combining urea and Optigen resulted in positive effects on the MN flow and DE of the diet, but apparently these advantages are observed only when there is a certain proportion of starch:ADF in the diet.

(Key Words: Slow-release urea, Finishing diets, Steers, Digestion, Microbial nitrogen)

INTRODUCTION

It is well known that, ruminal microbial protein synthesis (MPS) is the most important process in ruminant nitrogen (N) metabolism, since MPS not only contributes more than 50% of the amino acids absorbed in the small intestine, but also has an amino acid composition similar to that of the proteins required for meat production (Zinn and Owens, 1983, NRC, 1985, Seo et al., 2013). The N retention in the rumen is mainly mediated by the rate of degradation of N compounds and carbohydrates and by the energy available for the process of protein synthesis. It has been observed that in high-grain diets (ratio of starch *vs*. acid detergent fibre greater than 5.5 to 1) urea can be supplemented at 50% higher than recommended with positive effects on growth performance or in dietary energy utilization (Milton et al., 1997, Zinn et al., 2003). The latter can be partially explained by the possible synchrony of ruminal degradation rates between urea and starch. Currently, as a result of the cost of corn grain, the replacement of corn grain by dried distillers grain with solubles (DDGS) in feedlot diets is a common practice (Klopfenstein et al., 2008). Although the energy value of DDGS is similar to steam flaked corn (NRC, 1996), DDGS are lower in starch content (<6%) and higher in their content (>30%) of digestible fibre (Rosentrater, 2012; Carrasco et al., 2013). Therefore, depending on the replacement level, the fibre content in finishing diets can be increased up to two-fold [from 8 to 15% of total acid detergent fibre (ADF)] and the total starch content can be decreased by 20% (from 50 to

40%). Hypothetically, combining feed grade urea with slow-release urea in finishing diets should promote the synchrony between starch (high-rate of digestion) and fibre (low-rate of digestion), promoting better microbial protein synthesis and increases in ruminal digestion and the digestible energy of the diet. The beneficial effects of the supplementation of slow-release urea has been extensively studied in cattle that were fed a high-forage diets, i.e. rations for dairy and growing cattle (Inostroza et al., 2010, Alvarez et al., 2011); however, no research has examined the role of the starch:fibre ratio of the finishing diets on the effects of the combination of both sources of urea. Therefore, the purpose of this study was to evaluate the effects of combining feed grade urea and a slow-release urea product (OPT) on the characteristics of digestion, microbial protein synthesis and digestible energy in steers fed diets with different starch:ADF ratios.

MATERIALS AND METHODS

The trial was conducted at the Ruminant Metabolism Experimental Unit of the Instituto de Investigaciones en Ciencias Veterinarias of the Universidad Autónoma de Baja California located 10 km south of Mexicali City in northwestern México (32° 40' 7”N and 115° 28' 6”W). The area is about 10 m above sea level, and has Sonoran desert conditions (BWh classification according to Köppen). All animal management procedures were conducted within the guidelines of locally-approved techniques for animal use and care (NOM-051-ZOO-1995: humanitarian care of animals during mobilization of animals; NOM-062-ZOO-1995: technical specifications for the care and use of laboratory animals. Livestock farms, farms, centres of production, reproduction and breeding, zoos and exhibition hall, must meet the basic principles of animal welfare; NOM-024-ZOO-1995: animal health stipulations and characteristics during transportation of animals. These regulations are in accordance with the principles and specific guidelines presented in the

Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Animals, diets and sampling

Four Holstein steers (213 ± 4 kg) with ruminal and duodenal cannulas (Zinn and Plascencia, 1993) were used in a 4 × 4 Latin square design to examine the effect of the combination of Optigen (a polymer-coated urea; Optigen, Alltech Mexico, Guadalajara, Jalisco) and U in a finishing diet containing different starch:acid detergent fibre ratios (S:F) on the characteristics of digestion, microbial protein synthesis and digestible energy of diet. For the latter, three S:F ratios (3.0, 4.5 and 6.0) were tested using a combination of U (0.80%) and Optigen (1.0%). Additionally, a treatment of 4 S:F ratio with urea (0.80% in ration) as sole source of NPN was used to compare de effect of urea combination at same S:F ratio. For the above, four dietary treatments were compared:

1) Diet formulated to supply 2.14 Mcal/kg of NE_m with a S:F ratio of 4.5, containing 0.80% conventional urea as the sole source of NPN (U-4.5)
2) Diet formulated to supply 2.14 Mcal/kg of NE_m with a S:F ratio of 6.0, containing 0.80% conventional urea plus 1.00% OPT (OPT-6)
3) Diet formulated to supply 2.07 Mcal/kg of NE_m with a S:F ratio of 4.5, containing 0.80% conventional urea plus 1.00% OPT (OPT-4.5)
4) Diet formulated to supply 2.00 Mcal/kg of NE_m with a S:F ratio of 3.0, containing 0.80% conventional urea plus 1.00% of OPT (OPT-3)

The S:F ratio in the diet was manipulated by replacing the steam-flaked corn grain by dried distillers grain with solubles (DDGS) and forage (sudangrass hay and wheat straw) to reach S:F ratios of 3.0, 4.5 or 6.0 (Table 1). Chromic oxide (used as a source of

chromium to estimate nutrient flow and coefficient of digestion) was added to the diets (3.5 g/kg of diet on an air dry basis) and it was premixed with minor ingredients (urea and mineral supplement composed of limestone and trace mineral salts) before incorporation into complete mixed diets. All steers received *ad libitum* access to the control diet (U-4.5) for 14 days before initiation of the trial. To avoid refusals once the experiment started, dry matter intake was restricted to 4.55 kg/d (90% of observed ad libitum intake of steers during the 14-d preliminary period). The slow-release urea product was hand-weighed using a precision balance (Ohaus, mod AS612, Pine Brook, NJ), and was added (top-dressed) in equal proportions to the diet at time of feeding. Diets were fed in two equal portions at 0800 and 2000 h daily. Animals were housed in individual pens (3.9 m^2) in an indoor facility with a concrete floor covered by a neoprene carpet, automatic waterers and individual feed bunks. Experimental periods consisted of a 17-d diet adjustment period followed by a 4-d collection period. During the collection period, duodenal and faecal samples were taken from all steers, twice daily as follows: d 1, 0750 and 1350 h; d 2, 0900 and 1500 h; d 3, 1050 and 1650 h; and d 4, 1200 and 1800 h. Individual samples consisted of approximately 500 mL of duodenal chyme and 200 g (wet basis) of faecal material. Samples from each steer and within each collection period were prepared for analysis. During the final day of each collection period, a ruminal sample (~ 500 mL) was obtained from each steer at 0, 2, 4 and 6 hours after feeding via the ruminal cannula. Ruminal fluid was taken from the ruminal ventral sac by vacuum pump (Cole Parmer Instrument, Vernon Hill, IL) using a Tygon tube (i.d. 0.95 cm; USP Lima, Ohio), and pH of fresh samples was determined (Orion 261S, Fisher Scientific, Pittsburgh, PA.). Upon completion of the trial, ruminal fluid was obtained from all steers and composited for isolation of ruminal bacteria via differential centrifugation (Bergen et al., 1968). The microbial isolate served as the

purine:N reference for the estimation of microbial N contribution to chyme entering the small intestine (Zinn and Owens, 1986).

Sample analysis and calculations

Feed, duodenal and faecal samples were subjected to the following analysis: DM (oven drying at 105°C until no further weight loss; method 930.15; AOAC, 2000); ash (method 942.05; AOAC, 2000), Kjeldahl N (method 984.13; AOAC, 2000); ADF (Van Soest et al., 1991); chromic oxide (Hill and Anderson, 1958); and starch (Zinn, 1990). In addition, gross energy (GE, using the adiabatic bomb model 1271; Parr Instrument Co., Moline, IL. USA) was determined for feed and faecal samples. Ammonia N (method 941.04; AOAC, 2000) and purines (Zinn and Owens, 1986) were determined in duodenal samples. Ether extract (method 920.39; AOAC, 2000), calcium (method 927.02; AOAC, 2000) and phosphorus (method 964.06; AOAC, 2000) were determined in feed samples.

Microbial organic matter (MOM) and microbial nitrogen (MN) leaving the abomasum were calculated using purines as a microbial marker (Zinn and Owens, 1986). Organic matter fermented in the rumen (OMF) was considered equal to the OM intake minus the difference between the amount of total OM reaching the duodenum and the MOM reaching the duodenum. Feed N escaping to the small intestine was considered equal to the total N leaving the abomasum minus ammonia-N and MN and, thus, includes any endogenous contributions.

Statistical design and analysis

The effects of the treatments on the characteristics of digestion in the cattle were analysed as a 4 × 4 Latin square design using the GLM procedure (SAS Inst. Inc., Cary, NC). The statistical model for the trial was as follows:

$Y_{ijk} = \mu + S_i + P_j + T_k + E_{ijk}$,

where: Y_{ijk} is the response variable, μ is the common experimental effect, S_i is the steer effect, P_j is the period effect, T_k is the treatment effect and E_{ijk} is the residual error. The response curves of the S:F ratio in OPT treatments were analysed with orthogonal polynomials. In addition, the urea combination (urea *vs.* OPT) at same S:F ratio (4.5) was compared using the Student's t-test**.** The analysis was carried out using SAS (SAS Inst., Inc., Cary, NC; Version 9.1). The ruminal pH values, which were recorded at 0, 2, 4 and 6 h post-feeding, were analysed with a linear mixed model for repeated measures in a Latin square design according to SAS (SAS Inst., Inc., Cary, NC; Version 9.1), with evaluation of the covariance structures: A, CS, AR (1), and the animal as random components.

RESULTS AND DISCUSSIONS

The ratios of starch:ADF determined in the laboratory reached at least 98% of the planned for each treatment (Table 1). The effects of treatment on ruminal pH are shown in Table 2. The ruminal pH observed for each treatment in the present study was slightly greater (3.7%) than predicted based on the diet formulation (NRC, 1996, Level 1). This may be because of the incorporation of 1.80% limestone in the diet (Table 1), which has a high ruminal buffering capacity (Russell et al., 1980; Haaland et al., 1982). Ruminal pH was not affected by urea combination. The S:F ratio did not affect ruminal pH at 0 and 2 h post-feeding but, at 4 and 6 h post-feeding, the ruminal pH decreased as the S:F ratio increased (linear, $p<0.05$). As consequence of the rapid ruminal fermentation of starch, diets that contain increasing amounts of starch (grains) tend to promote a lower ruminal pH than those that contain lower quantities of starch (Huntington, 1997). In this sense, the S:F ratio of 6.0 contained 6.8 and 8.2% more starch than S:F ratios 4.5 and 3.0, respectively. At equal S:F ratios, the ruminal pH was very similar ($p=0.98$) between the urea and OPT

treatments, averaging 6.26 *vs.* 6.22 for U-4.5 and OPT-4.5, respectively. This indicates that, although U-4.5 contained 3.3% more energy than OPT-4.5 (2.14 *vs.* 2.07 Mcal EN_m/kg), the S:F is the most important factor, rather than the energy level *per se,* that impacts the ruminal pH.

Treatment effects on the characteristics of ruminal and total tract digestion are summarized in Table 3. Urea combination did not modified ruminal digestion of OM, starch, ADF and feed N. The S:F ratio did not affect the ruminal digestion of OM, starch and feed N, however, ruminal ADF digestion decreased linearly (p=0.02) as the S:F ratio increased. It has been observed that the depression in fibre digestion is largely a function of ruminal anion concentration (Peters et al., 1992). Russell and Wilson (1996) observed that fibre digestion decreases because cellulolytic bacteria cannot adequately control their intracellular anion gradient as pH declines. Grant and Weidner (1992) further verified this concept *in vitro* by fermenting various forages in cultures of ruminal fluid wherein pH was controlled experimentally by means of citrate buffers. They observed that both the lag phase and the rate of fibre digestion were sensitive to sustained culture pH below 6.2. The ruminal pH with the OPT-6 treatment was consistently maintained below 6.0, while in the rest of the treatments ruminal pH remained above 6.2 (Table 2).

The flow of microbial N to the small intestine (quadratic effect, P <0.01) and ruminal microbial efficiency (flow of microbial N to the small intestine as a proportion of fermented OM; quadratic effect, p=0.03) were greater than the 4.5 S:F ratio within OPT treatments. In the same manner, compared to the control diet, at an equal S:F ratio (4.5), the urea combination increased ($p \leq 0.02$) the MN flow to the duodenum by 7.1% and the MN efficiency by 7.3%. Burroughs et al. (1975) proposed that microbial N flow to the small intestine was equivalent to 0.0166TDN, estimated as follows: 0.0166×TDN×DMI, kg/d.

According to the expected TDN values (NRC, 1996) and DM intakes of the experimental diets (Table 3), the predicted flow of microbial N to the small intestine would be 62.3, 65.02, 63.06, and 59.25 g/d with U-4.5, OPT-6, OPT-4.5 and OPT-3, respectively. The MN flows of the U-4.5 and OPT-3 treatments were very close to those expected (98%); however, surprisingly, the MN flow of treatment OPT-6 was 5.6% less than expected. The decreased response observed for OPTU-6 can be explained, in part, by the reduced ADF ruminal digestion observed for this S:F ratio. In contrast, the urea combination for an S:F ratio of 4.5 produced a flow of N 4.2% greater than expected, reflecting a positive synchrony for this proportion of starch and ADF in the diet. It has been observed that MN production, among others, is a result of synchronization between the rate of hydrolysis of carbohydrates, and the rate at which N-NH_3 is produced during the hydrolysis of N compounds in the rumen (NRC, 1985; Orskov, 1992). The difference in microbial production observed between U-4.5 and OPT-4.5 could be partially explained by the improved synchrony for the combination of the two urea sources in OPT-4.5.

The S:F ratio did not affect post-ruminal digestion of OM, starch, ADF or N, although the digestion of organic matter tended (linear effect, p=0.09) to decrease as the S:F ratio in the diet decreased. Irrespective of the proportion of starch and fibre in the diets, the combination of two urea sources improved post-ruminal N digestion by an average of 2.8% (p=0.02) with no difference in the digestion of other components evaluated.

The S:F ratio affected OM and ADF total tract digestion. As the ratio increased, OM digestion increased (linear effect, P<0.01), but ADF total tract digestion decreased (p=0.03). The reduction in total tract OM digestion as the S:F ratio decreased was expected, and was largely attributable to relative differences in the total tract digestion of ADF (36%) versus starch (99%). Compared to the control diet, the inclusion of OPT in the diets

increased the total tract apparent N digestion (2.8%, P<0.05). However, this effect may be more a function of the increased N content of the diet brought about by the replacements (Holter and Reid, 1959).

Consistent with the effects on total tract OM digestion, the S:F ratio affected the digestibility of GE. As the ratio decreased, the digestibility of GE also decreased (linear effect, P<0.01). According to the expected DE values (NRC, 1996), the predicted DE were 1.00, 1.00, 1.02 and 1.00 times the expected values for U-4.5, OPT-6, OPT-4.5 and OPT-3, respectively. Thus, at a ratio of 4.5, the digestible energy was improved by 2% over the expected (P=0.04) level. This improvement represents an increase of 0.077 Mcal/kg of digestible energy. If we consider that: 1) the contribution of digestible energy content in diets is mainly due to changes in the participation of corn and DDGS; 2) that the DDGS contains a similar energy concentration as corn (Depenbusch et al., 2008; Uwitze et al., 2010); and 3) the difference in the content of DDG plus corn between U-4.5 and OPT-4.5 represents 6.5% (the sum of corn and DDGS participation in U-4.5 is 73%, whereas for diet OPT-4.5 it is 66.5%, Table 1), then the 2% of energy improvement in OPT-4.5 treatment represents the equivalent increase of 4.3% [(0.117-0.04) / (0.117/6.5)] corn grain in the diet.

It is concluded that combining feed grade urea and Optigen resulted in positive effects on the MN flow and digestible energy of the diet, but apparently these advantages are observed only when there is a certain proportion (4.5) of starch:ADF in the diet. Either a higher or lower S:F ratio than 4.5 failed to offer advantages over any of the parameters evaluated. It is necessary to continue research on the conditions of the finishing diet so that it is possible to get the most out of it with the use of slow release urea.

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Table 1. Ingredients and composition of experimental diets

Item	Treatments			
	U-4.5	OPT-6.0	OPT-4.5	OPT-3.0
Ingredient composition, % DMB				
Steam flaked corn	58.00	68.00	60.00	55.00
DDGS	15.00	4.00	6.50	7.00
Sudangrass hay	6.00	6.00	10.00	12.00
Wheat straw	4.00	4.00	5.50	8.00
Urea	0.80	0.80	0.80	0.80
Optigen 1200[1]	----	1.00	1.00	1.00
Cane molasses	8.00	8.00	8.00	8.00
Bakery waste	3.50	3.50	3.50	3.50
Yellow grease	2.00	2.00	2.00	2.00
Trace mineral salt [2]	0.40	0.40	0.40	0.40
Limestone	1.80	1.80	1.80	1.80
Chromic oxide	0.35	0.35	0.35	0.35
NE concentration[3], Mcal/kg of DM basis				
EN_m, Mcal/kg	2.14	2.14	2.07	2.00
EN_g, Mcal/kg	1.48	1.48	1.41	1.34
Nutrient composition, % of DM[4]				
Crude protein	12.48	13.50	13.70	13.53
Starch	42.92	48.20	44.90	39.40
ADF	9.63	8.03	10.10	13.27
Calcium	0.73	0.78	0.80	0.73
Phosphorus	0.43	0.31	0.33	0.33

[1]Optigen-1200. Alltech de México, Guadalajara Jalisco. [2]Trace mineral salt contained: $CoSO_4$, 0.068%; $CuSO_4$, 1.04%; $FeSO_4$, 3.57%; ZnO, 1.24%; $MnSO_4$, 1.07%, KI 0.052%; and NaCl, 92.96%. [3] Based on tabular net energy (NE) values for individual feed ingredients (NRC, 2000) with the exception of supplemental fat, which was assigned NE_m and NE_g values of 6.03 and 4.79, respectively (Zinn, 1988). [4]Dietary composition was determined by analyzing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.

Table 2. Effects of treatments on ruminal pH taken at 0, 2, 4, and 6 h *post-feeding*

	Treatments[1]						S:F ratio	
Item	U-4.5	OPT-6	OPT-4.5	OPT-3	EEM	U-4.5 vs. OPT-4.5	Linear	Quadratic
Ruminal pH								
0 h	6.60	6.47	6.56	6.76	0.12	0.19	0.06	0.19
2 h	5.82	5.84	5.89	6.05	0.07	0.67	0.09	0.63
4 h	6.20	5.80	6.11	6.15	0.09	0.48	0.04	0.32
6 h	6.42	6.06	6.41	6.51	0.09	0.98	0.02	0.31

[1] U-4.5 = Diet formulated to supply 2.14 Mcal/kg of NE_m with a ratio of starch:FDA of 4.5, containing 0.80% of conventional urea as the sole source of NNP ; OPT-6= Diet formulated to supply 2.14 Mcal/kg of NE_m with a ratio of starch:FDA of 6.0, containing 0.80% of conventional urea plus 1.00% of Optigen; OPT-4.5=Diet formulated to supply 2.07 Mcal/kg of NE_m with a ratio of starch:FDA of 4.5, containing 0.80% of conventional urea plus 1.00% of Optigen, and OPT-3= Diet formulated to supply 2.00 Mcal/kg of NE_m with a ratio of starch:FDA of 3.0, containing 0.80% of conventional urea plus 1.00% of Optigen (OPT-3).

Table 3. Influence of treatments on characteristics of ruminal and total tract digestion in Holstein steers

	Treatments[1]						S:F ratio	
Item	U-4.5	OPT-6	OPT-4.5	OPT-3	SEM[2]	U-4.5 vs. OPT-4.5	Linear	Quadratic
Intake, g/d								
DM[3]	4664	4646	4637	4469	64	0.02	0.03	0.03
OM[4]	4403	4419	4387	4370	61	0.08	<0.01	0.31
Starch	1903	2239	2084	1761	27	<0.01	<0.01	0.01
ADF[5]	452	374	501	602	7	<0.01	<0.01	0.01
N[6]	93	100	102	101	1.7	<0.02	0.25	0.11
GE, Mcal/d[7]	21.69	21.53	21.36	21.01	0.39	0.01	0.01	0.10
Ruminal digestión, %								
OM	54.23	56.35	55.29	54.85	0.62	0.27	0.13	0.71
Starch	77.98	78.18	78.00	78.36	0.62	0.99	0.85	0.73
ADF	26.37	19.09	26.74	27.42	1.97	0.90	0.03	0.19
Feed N	66.22	67.02	68.38	65.50	1.10	0.15	0.29	0.12
Duodenal flow of N, g/d	94.18	98.20	101	98.47	2.7	<0.01	0.86	0.03
Dudodenal flow of MN, g/d[8]	61.06	61.58	65.70	60.60	2.01	<0.01	0.30	<0.01
MN efficiency[9]	23.24	24.19	25.07	23.48	0.44	0.02	0.22	0.03
Dudenal flow of NAN, g/d[10]	92.52	94.68	97.80	95.45	0.83	<0.01	0.54	0.04
N efficiency[11]	0.99	0.94	0.96	0.94	0.01	0.09	0.95	0.24
Postruminal digestión, % leaving abomasum								
OM	65.84	64.62	63.28	60.86	1.28	0.21	0.09	0.74
Starch	94.29	94.62	94.78	94.96	0.34	0.35	0.76	0.82
ADF	22.47	17.96	14.33	12.68	2.40	0.07	0.17	0.75
N	75.55	76.56	77.64	76.86	4.9	0.02	0.68	0.18
Total-tract digestión, %								

DM	77.65	78.89	76.81	75.10	0.57	0.28	<0.01	0.77
OM	78.92	80.39	78.59	76.26	0.64	0.70	<0.01	0.73
Starch	98.74	98.83	98.85	98.87	0.08	0.40	0.74	0.99
ADF	42.99	33.68	37.37	36.87	0.80	<0.01	0.03	0.09
N	75.28	77.08	77.75	77.48	0.64	<0.01	0.55	0.42
DE, %[12]	78.30	78.94	78.23	76.26	0.53	0.91	<0.01	0.33
DE diet, Mcal/kg	3.64	3.66	3.60	3.44	0.02	0.27	<0.01	0.10
Observed-to-expected DE	1.00	1.00	1.02	1.00	0.006	0.04	0.69	0.04

[1] U-4.5 = Diet formulated to supply 2.14 Mcal/kg of NE_m with a ratio of starch:FDA of 4.5, containing 0.80% of conventional urea as the sole source of NNP ; OPT-6= Diet formulated to supply 2.14 Mcal/kg of NE_m with a ratio of starch:FDA of 6.0, containing 0.80% of conventional urea plus 1.00% of Optigen; OPT-4.5=Diet formulated to supply 2.07 Mcal/kg of NE_m with a ratio of starch:FDA of 4.5, containing 0.80% of conventional urea plus 1.00% of Optigen, and OPT-3= Diet formulated to supply 2.00 Mcal/kg of NE_m with a ratio of starch:FDA of 3.0, containing 0.80% of conventional urea plus 1.00% of Optigen (OPT-3). [2]SEM = Standard error. [3] DM = Dry matter. [4]OM = Organic matter. [5] ADF = Acid detergent fiber. [6] N = Nitrogen. [7] GE = Gross energy. [8] MN = Microbial nitrogen.[9] Microbial efficiency is expressed as duodenal MN, g/kg OM fermented in the rumen. [10] NAN= non-ammonia N. [11]N efficiency is expressed as duodenal non-ammonia N, g/g N intake. [12] DE = digestible energy.

EXPERIMENTO II

Running title: Slow-release product in finishing diets to lambs

Effects of combining feed grade urea and a slow-release urea product on performance, dietary energetics and carcass characteristics of feedlot lambs fed finishing diets with different starch:acid detergent fibre ratios

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ABSTRACT

Forty lambs (36.65 ± 3 kg) were used to examine the effect of combining a feed grade urea (U) and a slow-release urea product (SRU, Optigen II) in a finishing diet containing different starch:acid detergent fibre ratios (S:F) on growth performance, dietary energetics and carcass characteristics. Three S:F ratios (3, 4 and 5) were tested using a combination of U and SRU. Additionally, a treatment of 4 S:F ratio with urea (0.8% in ration) as sole source of NPN was used to compare de effect of urea combination at same S:F ratio . There were no effects of the urea combination or S:F ratio on dry matter intake (DMI). Urea combination increased ($P \leq 0.04$) average daily gain (ADG), gain for feed (G:F) and apparent energy retention per unit DMI. As S:F ratio increased, ADG, G:F and Net energy of diet increased linearly ($P < 0.01$). Irrespective of the S:F ratio, the urea combination improved the observed-to-expected dietary ratio and apparent retention per unit DMI being maximal (quadratic effect, $P = 0.04$) at 4 S:F ratio. There were no effects of urea combination on carcass characteristics. As S:F ratio increased, carcass weight, dressing percentage and LM area were increased linearly ($P \leq 0.02$). Combining feed grade urea and Optigen II resulted in positive effects on growth performance and dietary energetics, but apparently the better responses are observed when there is a certain proportion (ratio = 4.0) of starch:acid detergent fibre in the diet.

Keywords: Slow-release urea; Finishing diets; Hairy lambs; Growth performance, Dietary energetics, Carcass

INTRODUCTION

One topic of interest in recent years in feedlots is the search for strategies that optimize nutrient synchrony between N and carbohydrates compounds in rumen in order to promote better nutrient utilization and energy efficiency, and as a strategy for reducing the risk of environmental pollution (Hristov *et al.* 2011). The N retention in the rumen is mainly mediated by the rate of degradation of N compounds and carbohydrates and by the energy available for the process of protein synthesis (Tedeshi *et al.,* 2002). It has been observed that in high-grain diets (ratio of starch *vs*. acid detergent fibre (ADF) greater than 5 to 1) urea can be supplemented at 50% higher than recommended with positive effects on growth performance or in dietary energy utilization (Milton *et al.,* 1997; Zinn *et al.,* 2003). The latter can be partially explained by the possible synchrony of ruminal degradation rates between feed grade urea and starch. On the other hand, cattle that were fed a high-forage diets (> 10% ADF, i.e. rations for dairy and growing cattle) the use of slow-release urea products has demonstrated benefits on the nutrient synchrony (Inostroza *et al.,* 2010; Alvarez *et al.,* 2011). Currently, as a result of the cost of corn grain, the replacement of corn grain by dried distillers grain with solubles (DDGS) in feedlot diets is a common practice (Klopfenstein *et al.,* 2008). Although the energy value of DDGS is similar to corn grain (NRC, 2007; Estrada-Angulo *et al.,* 2013), DDGS are lower in starch content (<6%) and higher in their content (>30%) of digestible fibre (Rosentrater, 2012; Carrasco *et al.,* 2013). Therefore, depending on the replacement level, the starch:fibre ratio in finishing diets can be decreased (i.e. from 5.0 to 3.0). In growing-finishing diets, the few studies conducted on this field have been focused on evaluating the effect of SRU in direct substitution of high-protein ingredients (Pinos-Rodríguez *et al.,* 2010; Bourg *et al.,* 2012; Lascano *et al.,* 2012) more than as a strategy to promote synchrony, and no research has examined the role of the starch:fibre ratio

of the finishing diets on the effects of the combination of both sources of urea on growth performance. Based on the hypothesis of that combination of feed grade urea with slow release urea product in finishing diets should promote the synchrony between starch (high-rate of digestion) and fibre (low-rate of digestion), the aim of this experiment was to examine the combination of feed grade urea (U) and a slow-release urea product (SRU, Optigen II) in a finishing diet containing different starch:acid detergent fibre ratios (S:F) on growth performance, dietary energetics, and carcass characteristics of hairy lambs.

MATERIALS AND METHODS

This experiment was conducted at the Universidad Autónoma de Sinaloa Feedlot Lamb Research Unit, located in the Culiacán, México (24° 46' 13"N and 107° 21' 14"W). Culiacan is about 55 m above sea level, and has a tropical climate. All animal management procedures were conducted within the guidelines of locally-approved techniques for animal use and care (NOM-051-ZOO-1995: humanitarian care of animals during mobilization of animals; NOM-062-ZOO-1995: technical specifications for the care and use of laboratory animals. Livestock farms, farms, centres of production, reproduction and breeding, zoos and exhibition hall, must meet the basic principles of animal welfare; NOM-024-ZOO-1995: animal health stipulations and characteristics during transportation of animals, and NOM-033-ZOO-1995: humanitarian care and animal protection during slaughter process).

Animals, Diet and Experimental Design

Fifty Pelibuey × Kathdin lambs were received at the research facility before initiation of the experiment. Upon arrival, the lambs were treated for parasites (Tasasel 5%, Fort Dodge, Animal Health, México) and injected with 1×10^6 IU vitamin A (Synt-ADE, Fort Dodge Animal

Health). Two weeks before the initiation of the experiment lambs were fed the control diet. Following a 2-week evaluation period, 40 lambs (36.65 ± 3 kg) were selected from the original group of 50 lambs for use in the study, based on the uniformity of weight and general condition. The S:F ratio in the diet was manipulated by partially replacing the corn grain and dried distillers grain with solubles (DDGS) by forage (wheat straw) and soybean meal to reach S:F ratios of 3, 4 or 5. The slow-release urea product used was Optigen II (SRU, Alltech Mexico, Guadalajara, Jalisco). Based on the hypothesis of that combination of feed grade urea with slow release urea in finishing diets should promote the synchrony between starch (high-rate of digestion) and fibre (low-rate of digestion), thus the combination of urea and SRU (as percentage of DM of diet) was performed based on S:F ratios as follows: 1) 0.80 U and 1.00% SRU for 3 S:F ratio (U+SRU3); 2) 0.80 U: 0.80% SRU for 4 S:F ratio (U+SRU4), and 3) 1.00 U and 0.80% SRU for 5 S:F ratio (U+SRU5). An additional treatment of 4 S:F ratio with 0.8% of urea (U4) as sole source of NNP was used to compare de effect of urea combination at same S:F ratio. Ingredients and chemical composition of dietary treatments are shown in Table 1. Upon initiation of the experiment, lambs were weighed before the morning meal (electronic scale; TORREY TIL/S: 107 2691, TOR REY Electronics Inc., Houston TX, USA), and assigned to one of five weight groupings in 20 pens, with two lambs per pen. Pens were 6 m^2 with overhead shade, automatic waterers and 1 m fence-line feed bunks.. Dietary treatments were randomly assigned to pens within blocks. Lambs were weighed before the morning meal on day 1 and day 56 (harvest). Lambs were allowed *ad libitum* access to dietary treatments. Daily feed allotments to each pen were adjusted to allow minimal (< 5%) feed refusals in the feed bunk. The amounts of feed offered and of feed refused were weighed daily. Lambs were provided fresh feed twice daily at 0800 and 1400 hours. Feed bunks were visually assessed between 0740 and 0750 hours each morning, refusals were collected and

weighed and feed intake was determined. Adjustments to, either increase or decrease daily feed delivery, were provided at the afternoon feeding. Feed and refusal samples were collected daily for DM analysis, which involved oven drying the samples at 105°C until no further weight loss occurred (method 930.15; AOAC, 2000).

Calculations

The estimations of dietary energetic and expected DMI were performed based on the estimated initial and final shrunk body weight (SBW), to convert to a SBW basis is assuming that SBW is 96% of full weight (CSIRO, 1990; Cannas *et al.,* 2004). Average daily gains (ADG) were computed by subtracting the initial BW from the final BW and dividing the result by the number of days on feed (**DOF)**. The efficiency of BW gain was computed by dividing ADG by the daily DMI. The estimation of expected DMI was performed based on observed ADG and SBW according to the following equation: expected DMI, kg/day = $(EM/NE_m) + (EG/EN_g)$, where EM (energy required for maintenance, Mcal/d) = $0.056 \times SBW^{0.75}$ (NRC, 1985), EG (energy gain, Mcal/d) = $0.276 \times ADG \times SBW^{0.75}$ (NRC, 1985), NE_m and NE_g are energy concentration of experimental diets (derived from tabular values based on the ingredient composition of the experimental diet; NRC, 1985), and SBW represent full BW × 0.96, Cannas *et al.,* 2004]. The coefficient (0.276) was estimated assuming a mature weight of 113 kg for Pelibuey × Kathdin male lambs (Canton and Quintal, 2007). Dietary NE was estimated by means of the quadratic

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2c}$$

Where, $x = NE_m$, a =-0.41EM, b = 0.877 EM + 0.41 DMI + EG, and c = -0.877 DMI (Zinn *et al.,* 2008).

Carcass Data

The hot carcass weights (HCW) were obtained from all lambs at time of harvest. After carcasses (with kidneys and internal fat included) were chilled in a cooler at -2 to 1°C for 48 h, the following measurements were obtained: 1) body wall thickness (distance between the 12th and 13th ribs beyond the ribeye, five inches from the midline of the carcass); 2) fat thickness perpendicular to the *m. longissimus thoracis* (LM), measured over the center of the ribeye between the 12th and 13th rib; 3) LM surface area, measure using a grid reading of the cross sectional area of the ribeye between 12th and 13th rib, and 4) kidney, pelvic and heart fat (KPH). The KPH was removed manually from the carcass, and then weighed and reported as a percentage of the cold carcass weight (USDA, 1982).

Statistical Analyses

Performance (gain, gain efficiency, and dietary energetics) and carcass data, were analyzed as a randomized complete block design. The experimental unit was pen. The MIXED procedure of SAS (SAS Inst., Inc., Cary, NC; Version 9.1) was used to analyze the variables. The fixed effect consisted of treatment, and pen as the random component. The response curves of the S:F ratio in SRU treatments were analysed with orthogonal polynomials. In addition, the effect of urea combination (urea *vs*. U+SRU) in diets with a S:F ratio of 4 was compared using the t-Student test. The analysis was carried out using SAS (SAS Inst., Inc., Cary, NC; Version 9.1). Contrasts were considered significant when the *P*-value was ≤ 0.05, and tendencies were identified when the P-value was > 0.05 and ≤ 0.10.

RESULTS AND DISCUSSIONS

There were no effects of the urea combination on DM intake (Table 1). The absence of the effects on feed intake as consequence of the combination of urea plus SRU have been observed previously in steers fed a finishing diet (Tedeshi *et al.,* 2002; Pinos-Rodríguez *et al.,* 2010; Castañeda-Serrano *et al.,* 2013), and in dairy cattle which were fed with a 52:48 forage:concentrate diet (Galo *et al.,* 2003). In the same manner, the S:F ratio did not affect DM intake. In high-energy diets, ME intake, rather than physical fill, appeared to be the dominant factor influencing the DMI. Lu and Potchoiba (1990) observed a curvilinear response in goats when comparing 3 levels of energy (1.66, 1.86 and 2.06 Mcal NE_m/kg DM) in diets. However, consistent with our results, other studies (Mahgob *et al.,* 2000; Sheridan *et al.,* 2000; Loe *et al.,* 2004) did not found effect on DMI in finishing lambs when compare diets from 1.90 up to 2.16 Mcal NE_m/kg, which is similar to the range of energy density for the three S:F ratio treatments used in the present experiment (Table 1).

Urea combination increased ($P \leq 0.04$) average daily gain (ADG, 15.5%), gain for feed (G:F, 8.7%) and apparent energy retention per unit DMI (6.5%). Combining conventional urea with slow-release urea have been reported that promote improves on milk production (Akay *et al.,* 2004). Changes on productivity and/or energy efficiency can be explained by improves in N retention (decreases on ruminal ammonia concentration and increases on the flow of microbial to duodenum). It has been observed that in high-grain diets (ratio of starch *vs*. acid detergent fibre greater than 5 to 1) urea can be supplemented 50% higher than recommended with positive effects on growth performance or in dietary energy utilization (Milton *et al.,* 1997; Zinn *et al.,* 2003). The latter can be partially explained by the possible synchrony of ruminal degradation rates between urea and starch in those studies. López-Soto *et al.,* (2013) showed that steers fed a

combination urea and slow-release urea (using the same source of SRU) in S:F ratio of 4.5 had higher (P=0.04) flows of microbial N and digestible energy of diet than those fed urea, or than those fed urea plus SRU in diets with 3 and 6 S:F ratios. In previous studies conducted with steers (Tedeshi *et al.,* 2002; Pinos-Rodríguez *et al.,* 2010), urea combination did not affect growth performance or digestibility of the diet. Based on the experimental diets of the study of Tedeshi *et al.* (2003), the estimated S:F ratio of their experimental diets was 13.9, while on the study conducted by Pinos-Rodríguez *et al.* (2010) the estimated S:F ratio of the diets utilized was 5.4. Thus, the high S:F ratios of the diets used in the studies conducted by Tedeshi *et al.* (2002) and by Pinos-Rodriguez *et al.* (2010) could be a factor to the absence of effects on performance and feed efficiency of steers fed urea combination.

As S:F ratio increased, ADG, G:F and apparent retention per unit DMI increased linearly (P<0.01). Increases in feed efficiency have been a common response when comparing high-energy and low-energy diets (Kioumarzi *et al.,* 2008; NRC, 2007; Adbel-Basset, 2009). However, the effects of increased diet energy levels on ADG have been less consistent. In some instances (Lu and Potchoiba, 1990; García *et al.,* 2003), increasing the energy level had no effect on the ADG, whereas in others (Kioumarzi *et al.,* 2008; Adbel-Basset, 2009), an increase in energy level markedly increased the ADG. The latter could be explained by the relationship between DMI and the dietary energy density (Cannas *et al.,* 2004).

Across the entire 56 day period, the average observed-to-expected DMI of controls was 102% of the expected value, based on tabular (NRC, 2007) estimates of diet energy density and observed SBW and ADG values (Table 2), supporting the practicality of the prediction equations proposed by the NRC (1985) for the estimation of DMI in relation to SBW and ADG in feedlot lambs. In the same manner, the observed-to-expected NE diet averaged 0.98. There were no

effect of S:F ratio on observed-to-expected NE of diet. Irrespective of the S:F ratio, the urea combination improved the observed-to-expected dietary ratio and apparent retention per unit DMI being maximal (quadratic effect, P=0.04) at 4 S:F ratio. According to the expected NE values (NRC, 2007), the predicted NE were 1.02, 1.04, 1.08 and 1.05 times the expected values for controls, U+SRU3, U+SRU4, and U+SRU5, respectively. Thus, comparing with the rest of treatments, at a S:F ratio of 4, the digestible energy in U+SRU4 treatment was improved an average of 4% over the expected. Considering the same diet composition between control (U4) and U+SRU4 treatment (Table 1), then comparing with the control diet, the energy improvement in U+SRU4 treatment represent the equivalent of increases of an 5.5% [2.15-2.03) /2.2]corn grain in the diet. This could supports the theory that the S:F ratio is most important factor, rather than energy level *per se* that impact on the synchrony when urea and SRU are combined.

The treatments effects on the carcass characteristics are shown in table 3. Consistent with previous reports in steers (Pinos-Rodríguez *et al.*, 2010; Holland and Jennings, 2011) urea combination that replace soybean meal did not affect carcass characteristics. The linear increases in HCW and dressing percentage as result of increased on S:F ratio is likely due to concomitant linear increase in ADG (Block *et al.*, 2001). In the same manner, increased LM area has been a consistent response to increased rate of ADG (Zinn *et al.*, 2007).

It is concluded that combining urea and slow-release urea product (Optigen II) resulted in positive effects on growth performance and dietary energetics, but apparently the better responses are observed when there is a certain proportion (S:F ratio=4) of starch:ADF in the diet, when the S:F ratio increases or decreases, the level of response decreases. Further studies are

needed to determine on the conditions of the finishing diet so that it is possible to get the most out of it with the use of slow release urea.

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Table 1. Ingredients and composition of experimental diets.

Item	Treatments			
	U4	U+SRU3	U+SRU4	U+SRU5
Ingredient composition, % DMB				
Steam flaked corn	60.00	55.00	60.00	65.00
DDGS	8.00	6.00	8.00	13.00
Soybean meal	5.00	5.00	4.00	0.00
Wheat straw	12.00	18.00	12.00	6.00
Urea	0.80	0.80	0.80	1.00
Optigen 1200[1]	----	1.00	0.80	0.80
Cane molasses	9.70	9.50	9.60	9.40
Yellow grease	2.20	2.50	2.50	2.50
Trace mineral salt [2]	0.50	0.50	0.50	0.50
Limestone	1.80	1.70	1.80	1.80
NE concentration[3], Mcal/kg of DM basis				
EN_m, Mcal/kg	2.00	1.89	1.99	2.10
EN_g, Mcal/kg	1.34	1.26	1.34	1.43
Nutrient composition, % of DM[4]				
Crude protein	14.01	15.70	15.40	15.84
Starch	42.62	38.77	42.10	45.12
ADF	10.71	13.07	10.52	8.53
Calcium	0.78	0.76	0.80	0.79
Phosphorus	0.35	0.32	0.35	0.41

[1]Optigen-1200. Alltech de México, Guadalajara Jalisco.

[2]Trace mineral salt contained: $CoSO_4$, 0.068%; $CuSO_4$, 1.04%; $FeSO_4$, 3.57%; ZnO, 1.24%; $MnSO_4$, 1.07%, KI 0.052%; and NaCl, 92.96%.

[3] Based on tabular net energy (NE) values for individual feed ingredients (NRC 2007) with the exception of supplemental fat, which was assigned NE_m and NE_g values of 6.03 and 4.79, respectively (Zinn 1988).

[4]Dietary composition was determined by analyzing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.

Table 2. Influence of treatments on growth performance and dietary energy of lambs[1].

Item	Treatments[1]				SEM	U4 vs. U+USR4	S:F ratio[2]	
	U4	U+SRU3	U+SRU4	U+SRU5			Linear	Quadratic
Pen replicates	5	5	5	5				
Days on feed	56	56	56	56				
Weight, kg [3]								
Initial	36.61	36.49	36.75	36.73	0.21	0.66	0.42	0.60
Final	49.89	49.30	52.34	52.42	0.64	0.02	<0.01	0.09
Average daily gain, kg	0.235	0.229	0.278	0.280	0.013	0.04	0.02	0.15
Dry matter intake, kg	1.237	1.257	1.335	1.295	0.046	0.16	0.57	0.31
Gain for feed, kg/kg	0.190	0.180	0.208	0.216	0.006	<0.01	<0.01	0.03
Dietary net energy, Mcal/kg[4]								
Maintenance	2.03	1.98	2.15	2.21	0.02	0.01	<0.01	0.03
Gain	1.37	1.33	1.48	1.53	0.02	0.01	<0.01	0.03
Observed to expected dietary ratio[5]								
Maintenance	1.02	1.04	1.08	1.05	0.01	<0.01	0.42	0.03
Gain	1.02	1.05	1.10	1.06	0.01	<0.01	0.60	0.02
Observe to expected daily DM intake[6]	0.98	0.94	0.90	0.94	0.01	<0.01	0.57	0.01

[1] U4=0.80% U for 4 S:F ratio, U+SRU3 = 0.80 U and 1.00% SRU for 3 S:F ratio, U+SRU4= 0.80 U: 0.80% SRU for 4 S:F ratio, and U+SRU5= 1.00 U and 0.80% SRU for 5 S:F ratio.

[2] Proportion of starch *vs.* fibre acid detergent in diet.

[3] The initial BW was reduced by 4% to adjust for the gastrointestinal fill, and all lambs were fasted (food but not drinking water was withdrawing) for 18 h before recording the final BW.

[4] The estimation of dietary NE was performed based on observed ADG, DMI and average shrunk weight (SBW) and was estimated by means of the quadratic formula: $x = (-b \pm \sqrt{b^2 - 4ac})/2c$, where $x = NE_m$, a =-0.41EM, b = 0.877 EM + 0.41 DMI + EG, and c = -0.877 DMI, where EM= maintenance coefficient of 0.056 Mcal/$BW^{0.75}$ (NRC, 1985), EG is the daily energy deposited (Mcal/day) estimated by equation: EG= [(0.276 × ADG) × $SBW^{0.75}$; NRC, 1985], and DMI is the average daily dry matter intake (Zinn et al., 2008).

[5]Observed to expected dietary net energy (NE) ratio was computed by dividing NE observed between expected diet NE, which was estimated based on tabular values for individual dietary ingredients (NRC, 2007).

[6]Expected DMI was performed based on observed ADG, average shrunk weight (SBW) and the calculated NE diet and was computed as follows: DMI, kg/day= (EM/NEm) + (EG/ENg), where EM= maintenance coefficient of 0.056 Mcal/$BW^{0.75}$ (NRC 1985) and EG is the daily energy deposited (Mcal/day) estimated by equation: EG= [(0.276 × ADG) × $SBW^{0.75}$, NRC 1985]. The divisors NE_m and NE_g are the NE of diet [Table 1, calculated from tables of composition of feed (NRC 2007)].

Table 3. Treatment effects on carcass characteristics.

	Treatments[1]						S:F ratio[2]	
Item	U4	U+SRU3	U+SRU4	U+SRU5	SEM[2]	U4 vs. U+SRU4	Linear	Quadratic
Hot carcass weight, kg	29.79	28.45	30.97	31.16	0.43	0.08	<0.01	0.05
Cold carcass weight, kg	29.44	28.13	30.68	30.83	0.42	0.06	<0.01	0.04
Drip loss, %	1.18	1.03	0.95	1.09	0.16	0.34	0.80	0.59
Dressing percent	59.66	57.66	59.17	59.44	0.45	0.46	0.02	0.28
Longissimus muscle área, cm^2	15.21	14.63	14.88	16.82	0.51	0.65	0.02	0.20
Backfat thickness, mm	2.52	2.53	2.66	2.61	0.25	0.69	0.82	0.78
Kidney-pelvic fat, %	2.84	2.56	2.90	3.08	0.21	0.85	0.09	0.73
Body wall thickness, mm	13.81	13.42	13.43	13.81	0.50	0.61	0.59	0.77

[1] U4=0.80% U for 4 S:F ratio; U+SRU3 = 0.80 U and 1.00% SRU for 3 S:F ratio; U+SRU4= 0.80 U: 0.80% SRU for 4 S:F ratio, and U+SRU5= 1.00 U and 0.80% SRU for 5 S:F ratio.
[2] Proportion of starch *vs.* fibre acid detergent in diet.

EXPERIMENTO III

Running title: Slow-release product in finishing diets to feedlot cattle

Effects of a combining feed grade urea and a slow-release product on performance, dietary energetics and carcass characteristics of steers fed finishing diets

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Abstract

Recent findings have shown that microbial nitrogen flow and digestible energy of diet are increased when urea (U) is combined with a slow-release urea product (SRU) in diets with a starch:fibre (S:F) ratio of 4.5, while feed grade urea has shown greater effects on growth performance or dietary energy utilisation when the diet contains a S:F ratio of greater than 5.0. These results can be partially explained by the better synchronization of ruminal degradation rates between those urea sources with the carbohydrates of the diets. Therefore, 60 crossbreed steers (372.4±15 kg) were used to evaluate the effects of combining U and SRU in a diet with a S:F ratio of 4.5 vs. urea that was supplemented in diets with different S:F ratios (3.5, 4.5, and 5.5) on growth performance, dietary energetics, and carcass characteristics. Urea combination did not affect average daily gain (ADG), but reduced dry matter intake (DMI, as % of body weight [BW]) enough to increase feed efficiency (G:F) and dietary net energy (NE). As the S:F ratio increased, the DMI, ADG, G:F, and NE of diet increased linearly. Irrespective of the S:F ratio, urea diets did not modify the observed-to-expected NE ratio nor the apparent retention per unit DMI, while urea combination increased by 7.2% and 8.4%, respectively, the observed-to-expected dietary ratio and the apparent retention per unit DMI. Urea combination had no effect on carcass characteristics. As the S:F ratio increased, carcass weight and LM area were increased linearly. Combining feed grade urea and SRU in diets with a 4.5 starch:fibre ratio resulted in positive effects on the efficiency of utilisation of dietary energetics.

Key words: slow-release urea; finishing diets; steers; Optigen; dietary energetics; growth performance; carcass

1. Introduction

Because of its low cost per unit of N compared with most sources of natural protein, urea (U) is typically the primary source of supplemental N in conventional steam-flaked corn-based finishing feedlot diets (Vasconcelos et al. 2009). Previous reports (Milton et al. 1997; Zinn et al. 2003) have shown that supplemental U has more positive effects on growth performance or dietary energy utilisation when the diet contains a starch:acid detergent fibre (ADF) ratio of greater than 5.0. However, as a result of the cost of grains, the replacement of grains by co-products (i.e., DDGS) in feedlot diets is a common practice (Klopfenstein et al. 2008). This change produces diets that contain a lower amount of starch and a greater amount of fibre (Carrasco et al. 2013). Thus, the S:F ratio in finishing diets can be reduced (i.e., from 5.0 to 3.0). Hypothetically, combining feed grade U with slow-release urea (SRU) in this type of diet should elicit a better synchrony between starch (high rate of digestion) and fibre (low rate of digestion). Recent findings (López-Soto et al. 2013) indicate that the combination of U and SRU when there is a certain proportion (4.5 to 1) of starch:ADF in the diet results in greater improvements in the microbial nitrogen flow and digestible energy of the diet. Because no information is available related to the growth performance and dietary energetics of finishing cattle to verify the findings of Lopez et al. (2013), the aim of this experiment was to examine the effects of the supplementation of U and SRU in a diet with a S:F ratio of 4.5 vs. U supplementation in diets with different S:F ratios (3.5, 4.5, and 5.5) on growth performance, dietary energetics, and carcass characteristics.

2. Material and Methods

All animal management procedures were conducted within the guidelines of locally-approved techniques for animal use and care (NOM-051-ZOO-1995: humanitarian care of animals during mobilisation of animals; NOM-062-ZOO-1995: technical specifications for the care

and use of laboratory animals. Livestock farms, farms, centres of production, reproduction and breeding, zoos and exhibition halls, must meet the basic principles of animal welfare; NOM-024-ZOO-1995: animal health stipulations and characteristics during the transportation of animals).

2.1 Animal processing, housing, and feeding

Sixty crossbreed steers (live weight average 372.4±15 kg) were used to evaluate the treatments effects on characteristics of growth-performance, dietary energetic, and carcass characteristics. The experiment lasted 73 days. The treatment 1 consisted in combining urea and slow-release urea product (0.80% each one on DM basis) supplemented in a diet with 4.5 S:F ratio, while the treatments 2, 3 and 4 were formulated by the supplementation of 0.8% of urea solely in diets with 3.5, 4.5 or 5.5 S:F ratio. The S:F ratio in the diet was manipulated by partially replacing the corn grain by sudangrass hay (Table 1). The slow-release urea product used was Optigen II (SRU; a polymer-coated urea, Optigen, Alltech Mexico, Guadalajara, Jalisco). Six weeks before initiation of the experiment steers were vaccinated for bovine rhinotracheitis and parainfluenza 3 (TSV-27, Pfizer Animal Health, México), clostridials (Fortress 7, Pfizer Animal Health, Mexico), and Pasteurella haemolytica (One Shot, Pfizer Animal Health, México), and treated for parasites (CYDECTIN® NF, Pfizer Animal Health, México; Trodax, Merial, México). Steers were injected with 1×10^6 IU vitamin A (Vita-Jec A&D "500", Synt-ADE®, Fort Dodge, Animal Health, México) and were implanted with 200 mg of trenbolone acetate and 20 mg of estradiol 17β (Revalor H®, Intervet, México). Steers were blocked by weight into five blocks and assigned within blocks to 20 pens (3 steers/pen). Pens were 4.00× 8.20 m with 19 m^2 of shade, and were equipped with automatic waterers and fence-line feed bunks (2.37 m in length). Diets were prepared at weekly intervals. Daily feed allotments to each pen were adjusted to allow minimal (< 5%) feed refusals in the feed bunk. The amounts of feed offered and of feed refused

were weighed daily. Steers were provided fresh feed twice daily at 0800 and 1400 hours. Feed bunks were visually assessed between 0700 and 0730 hours each morning, refusals were collected and weighed and feed intake was determined. Adjustments to, either increase or decrease daily feed delivery, were provided at the afternoon feeding. Feed and refusal samples were collected daily for DM analysis, which involved oven drying the samples at 105°C until no further weight loss occurred (method 930.15, AOAC 2000). In addition, Kjeldahl N (method 984.13, AOAC 2000), ADF (Van Soest et al. 1991), and starch (Zinn 1990) were determined in feed samples

2.2 Calculations

The estimations of expected DMI and dietary energetic were performed based on measures of initial and final shrunk body weight (SBW), assuming that SBW is 96% of full weight (NRC 1996). Average daily gains (ADG) were computed by subtracting the initial BW from the final BW and dividing the result by the number of days on feed. The efficiency of BW gain was computed by dividing ADG by the daily DMI. The estimation of expected DMI was performed based on the observed ADG and SBW according to the following equation: expected DMI, kg/day = (EM/NE_m) + (EG/EN_g), where EM (energy required for maintenance, Mcal/day) = $0.077W^{0.75}$ (Garrett 1971), EG =$ADG^{1.097} \times 0.0557W^{0.75}$ (NRC 1984), NE_m and NE_g are 2.22 and 1.55 Mcal/kg, respectively (derived from tabular values based on the ingredient composition of the experimental diet; NRC 1996). The dietary NE_g was derived from NE_m by the equation: $NE_g = 0.877\ NE_m - 0.41$ (Zinn et al. 2008). Dry matter intake is related to energy requirements and dietary NE_m according to the equation: $DMI = EG/(0.877NE_m - 0.41)$, and can be resolved for estimation of dietary NE by means of the quadratic formula: $x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2c}$, where $x = NE_m$, a =-0.41EM, b = 0.877 EM + 0.41 DMI + EG, and

c = -0.877 DMI (Zinn and Shen 1998).

2.3 Carcass data

All steers were harvested on the same day. Hot carcass weights (HCW) were obtained from all heifers at the time of slaughter. After carcasses were chilled for 48 h, the following measurements were obtained: 1) LM area, taken by direct grid reading of the muscle at the 12th rib taken at a location three-quarters of the length laterally from the backbone end; 2) subcutaneous fat over the ribeye muscle at the 12th rib taken at a location three-quarters of the lateral length from the chin bone end; 3) kidney, pelvic and heart fat (KPH) as a percentage of carcass weight, and 4) marbling score (USDA 1997).

2.4 Statistical analyses

Performance (gain, gain efficiency, and dietary energetics) and carcass data were analysed as a randomised complete block design. The experimental unit was the pen. The MIXED procedure of SAS (SAS Institute 2004) was used to analyse the variables. The fixed effect consisted of treatment, and pen as the random component. To compare the effect of urea combination *vs*. urea at same S:F ratio (4.5) the t-Student test was used. The response curves of the S:F ratio in U treatments were analysed with orthogonal polynomials. The analysis was carried out using SAS (SAS Inst., Inc., Cary, NC; Version 9.1). Contrasts were considered significant when the *P*-value was ≤ 0.05, and tendencies were identified when the *P*-value was > 0.05 and ≤ 0.10.

3. Results and Discussion

According to the determinations of starch and ADF obtained in the laboratory, the starch:ADF ratio reached 101, 0.99, 102, and 101% of the planned for each treatment (Table 1). Treatment effects on growth performance of feedlot steers are shown in Table 2. U combination did not affect (*P*=0.96) average daily gain (ADG), but tended to reduce dry matter intake (DMI; 5.1%,

P=0.06), and reduced DMI expressed as a percentage of live weight (5.8%, *P*=0.02). In a few studies, the absence of effects on feed intake of the combination of U plus SRU products has been observed previously in steers fed a finishing diet (Tedeshiet al. 2002; Pinos-Rodríguez et al. 2010; Castañeda-Serrano et al. 2013). However, a tendency for reduction in DMI has been observed in steers fed diets containing 2.25% of a solution of SRU product based on calcium bond urea (Duff et al. 2000). Taylor-Edwards et al. (2009) reported a 4.4% reduction in DMI when 0.8% of Optigen II replaced 0.8% of urea, but these responses were noted only in the last 28 days of the 56 days of the experiment. The basis for the inconsistencies in DMI responses to SRU supplementation is not certain, but may be related to the taste of SRU products and/or diet composition. In the present experiment, the decreases in DMI on SRU+U treatment was enough to increase feed efficiency (G:F) by 14.2% (*P*= 0.02) and to increase the dietary net energy (NE) by 7.2%. Duff et al. (2000) reported that the gain-to-feed ratio was improved by 4.4% (*P*<0.01) when 100% of U (1.21% in the diet) and 100% of soybean meal (2.80% in the diet) were replaced by 2.25% of Ruma Pro (a SRU product) plus 1.76% of corn grain. Changes in the productivity and/or energy efficiency of cattle that have been fed diets containing SRU can be explained by improvements in N retention by decreases in ruminal ammonia concentration and increases in microbial flow to the duodenum (Akay et al. 2004; Alvarez-Almora et al. 2011). López-Soto et al.(2013) showed that steers fed a combination of U and SRU(Optigen) in a diet with a S:F ratio of 4.5 had higher (*P*=0.04) flows of microbial N and digestible energy of diet than those fed U and those fed U plus SRU in diets with a S:F ratio of 3 or 6. They explained that the combination of feed grade U with SRU in diets containing a certain ratio of starch:fibre should promote a better synchrony between starch (high rate of digestion) and fibre (low rate of digestion). In contrast, other studies (Tedeshi et al. 2003; Pinos-Rodríguez et al. 2010) showed

that SRU supplementation to finishing steers did not have positive effects on gain nor feed efficiency. The estimated S:F ratio of the experimental diets of studies conducted by Tedeshi et al. (2002) and by Pinos-Rodríguez et al. (2010) was over 5.4; thus, the high S:F ratios of the diets used in those studies could be a factor in the absence of effects on the performance and feed efficiency of steers fed a combination of U and SRU.

As the S:F ratio increased, the DMI, ADG, G:F, and NE of diet increased ($P \leq 0.03$) linearly. The lack of a decrease in DMI with reduced forage level observed in the present experiment was merely apparent. The average observed DMI of steers fed U diets was 102% of the expected based on tabular (NRC 1996) estimates of diet energy density and observed shrunk body weight (SBW) and ADG (Table 2), supporting the practicality of the prediction equations proposed by the NRC (1996) for the estimation of DMI in relation to SBW and ADG in feedlot cattle. Still, similar responses in DMI between diets containing different levels of forage have been observed previously in trials involving steam-flaked corn-based diets (Zinn et al. 1994; Calderon-Cortes and Zinn 1996).On the other hand, the increases in gain, feed efficiency, or both, as a result of increases in energy density in diets are well documented (Zinn et al. 2008).

Irrespective of the S:F ratio, U diets did not modify the dietary energy ratio nor the observed-to-expected DMI. It has been observed that in high-grain diets (a starch:ADF ratio of greater than 5.0:1), U can be supplemented at a level 50% higher than the recommended with positive effects on growth performance or in dietary energy utilisation (Milton et al. 1997; Zinn et al. 2003). One possible advantage to higher U levels in finishing diets might be related to the buffering effects of U as a result of its hydrolysis to CO_2 and NH_3 and the potential buffering effects via ammonia (Galyean 1996), and/or because the synchrony of ruminal degradation rates between feed grade U and starch is maybe more favourable in these types of diets. The observed-

to-expected dietary energy and intake are an important and practical application of current standards for energetics in nutrition research (Zinn et al. 2008). Based on diet composition and measures of growth performance, there is an expected energy intake, and hence, DMI (NRC 1996). The estimation of dietary energy and the ratio of observed-to-expected DMI reveals differences in efficiency independently of ADG, providing important insight into potential treatment effects on the efficiency of energy utilisation of the diet itself. In the present experiment, the absence of effects on observed-to-expected DMI and dietary NE of the urea treatments at different S:F ratios showed that starch and fibre at these proportions did not provide any energetic advantage when they were supplemented with U. Compared with the U diets, combining U and SRU at a 4.5 S:F ratio increased (P<0.01) by an average of 7.2% the observed-to-expected dietary ratio and reduced by 8.4% (P<0.01) the apparent retention per unit DMI. This corroborates the findings of Lopez et al. (2013), which reported an energetic advantage (increases in digestible energy) in cannulated steers when the combination of SRU+U was given at a ratio of starch and ADF identical to that used in the present experiment. In practical terms, if we consider that the diet composition of combined U treatment (SRU+U-4.5) and U treatment at the same S:F ratio (U-4.5, Table 1) were practically identical, the energy improvement observed for SRU+U-4.5 treatment represents the equivalent of an increase of 6.4% [(2.14– 2.00)/2.18] of steam-flaked sorghum in the diet.

Treatment effects on carcass characteristics are shown in Table 2. Similar to previous reports (Pinos-Rodríguez et al. 2010; Holland and Jennings 2011), there were no effects of U combination on carcass characteristics. As the S:F ratio increased, carcass weight and LM area were increased linearly. The linear increases in hot carcass weight and dressing percentage, as a result of increased S:F ratio, was likely due to the concomitant linear increase in ADG (Block et

al. 2001). In the same manner, an increased LM area has been a consistent response to an increased rate of ADG (Zinn et al. 2007).

4. Conclusions

Under the conditions of the current experiment, it was concluded that combining U with Optigen II in diets containing an approximate starch:ADF ratio of 4.5:1 increases by 8% the dietary energy efficiency. This energetic advantage represents the equivalent of a 6% increase of grain in the diet. An additional point is that the use of the combination of U and SRU as an alternative source of non-protein nitrogen for finishing diets in feedlots will depend on its cost and the relative prices of forage and grain.

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Table 1. Ingredients and composition of experimental diets

Item	Treatments			
	SRU+U-4.5	U-3.5	U-4.5	U-5.5
Ingredient composition, % DMB				
Steam flaked sorghum	61.00	56.00	61.00	66.00
DDGS	11.00	11.00	11.00	11.00
Sudangrass hay	12.00	18.00	12.00	8.00
Urea	0.80	0.80	0.80	0.80
Optigen II [a]	0.80	---	---	---
Cane molasses	9.83	9.63	10.63	9.63
Yellow grease	2.50	2.50	2.50	2.50
Trace mineral salt [b]	0.40	0.40	0.40	0.40
Limestone	1.67	1.67	1.67	1.67
NE concentration [c], Mcal/kg of DM basis				
EN_m, Mcal/kg	2.03	1.98	2.03	2.09
EN_g, Mcal/kg	1.38	1.34	1.38	1.43
Nutrient composition, % of DM [d]				
Crude protein (N×6.25)	15.90	13.57	13.71	13.84
Starch	42.36	39.56	42.40	46.38
ADF	9.31	11.36	9.24	8.36
Starch:ADF ratio	4.55	3.48	4.59	5.55

[a] Optigen-II. Alltech de México, Guadalajara Jalisco. [b] Trace mineral salt $CoSO_4$, 0.068%; $CuSO_4$, 1.04%; $FeSO_4$, 3.57%; ZnO, 1.24%; $MnSO_4$, 1.07%; KI, 0.052%; NaCl, 92.96%. [c] Based on tabular net energy (NE) values for individual feed ingredients (NRC, 2000) with the exception of supplemental fat, which was assigned NE_m and NE_g values of 6.03 and 4.79, respectively (Zinn, 1988). [d] Dietary composition was determined by analyzing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.

Table 2. Influence of treatments on growth performance and dietary energy of feedlot steers.

Item	Treatments[a]				SEM	SRU+U-4.5 vs. U-4.5	S:F ratio[b]	
	SRU+U-4.5[c]	U-3.5	U-4.5	U-5.5			Linear	Quadratic
Pen replicates	5	5	5	5				
Days on feed	73	73	73	73				
Weight, kg [d]								
Initial	371.6	372.2	372.7	372.9	3.3	0.81	0.89	0.96
Final	461.2	453.9	462.08	473.9	6.5	0.92	0.05	0.82
Average daily gain, kg	1.279	1.158	1.276	1.443	0.052	0.97	<0.01	0.97
Dry matter intake, kg	7.896	8.321	8.548	9.085	0.219	0.06	0.03	0.57
Dry matter intake, % LW	1.896	2.012	2.046	2.145	0.037	0.02	0.03	0.49
Gain for feed, kg/kg	0.162	0.139	0.149	0.159	0.003	0.02	<0.01	0.97
Dietary net energy, Mcal/kg[e]								
Maintenance	2.14	1.93	2.00	2.05	0.018	<0.01	<0.01	0.71
Gain	1.47	1.28	1.35	1.39	0.016	<0.01	<0.01	0.71
Observed to expected dietary ratio[f]								
Maintenance	1.06	0.98	0.99	0.98	0.009	<0.01	0.59	0.55
Gain	1.06	0.96	0.98	0.97	0.011	<0.01	0.59	0.55
Observe to expected daily DM intake[g]	0.94	1.02	1.02	1.04	0.011	<0.01	0.39	0.50

[a] SRU+U-4.5=0.80% U plus 0.80% at 4.5 S:F ratio, U-3.5 = 0.80 U at 3.5 S:F ratio, U-4.50=.80% U at 4.5 S:F ratio, and U-5.5= 0.80% U at 5.5 S:F ratio. [b] Proportion of starch *vs*. fibre acid detergent in diet. [c] Source of SRU was Optigen II, Alltech Inc., México, Guadalajara México. [d] The initial and BW was reduced by 4% to adjust for the gastrointestinal fill. [e] The estimation of dietary NE was performed based on observed ADG, DMI and average shrunk weight (SBW) and was estimated by means of the quadratic formula: $x = (-b \pm \sqrt{b^2 - 4ac})/2c$, where $x = NE_m$, a =-0.41EM, b = 0.877 EM + 0.41 DMI + EG, and c = -0.877 DMI, where EM= maintenance coefficient of 0.077 Mcal/$BW^{0.75}$ (NRC 1984), EG is the daily energy deposited (Mcal/day) estimated by equation: $EG=ADG^{1.097} \times 0.0557W^{0.75}$ (NRC 1984), and DMI is the average daily dry matter intake (Zinn et al. 2008). [f] Observed to expected dietary net energy (NE) ratio was computed by dividing NE observed between expected diet NE, which was estimated based on tabular values for individual dietary ingredients (NRC 1996). [g] Expected DMI, kg/day = $(EM/NE_m) + (EG/EN_g)$; where, NE_m and EN_g is the diet energy concentration.

Table 3. Treatment effects on carcass characteristics.

Item	Treatments[a]				SEM	SRU+U-4.5 vs. U-4.5	S:F ratio[b]	
	SRU+U-4.5	U-3.5	U-4.5	U-5.5			Linear	Quadratic
Hot carcass weight, kg	301.7	293.1	302.7	308.6	4.12	0.86	0.02	0.72
Cold carcass weight, kg	298.1	289.6	299.1	305.0	4.07	0.86	0.02	0.72
Drip loss, %	1.20	1.18	1.19	1.19	0.023	0.73	0.87	0.98
Dressing percent	62.80	62.08	62.90	62.57	0.32	0.78	0.81	0.28
Longissimus muscle área, cm^2	78.10	77.18	78.30	80.73	1.079	0.90	0.04	0.62
Backfat thickness, mm	0.62	0.62	0.61	0.67	0.065	0.94	0.61	0.70
Kidney-pelvic fat, %	1.93	2.00	2.00	2.13	0.154	0.76	0.55	0.73
Marbling score	3.27	3.20	3.28	3.33	0.157	0.95	0.55	0.95

[a] SRU+U-4.5=0.80% U plus 0.80% at 4.5 S:F ratio, U-3.5 = 0.80 U at 3.5 S:F ratio, U-4.50=.80% U at 4.5 S:F ratio, and U-5.5= 0.80% U at 5.5 S:F ratio.
[b] Proportion of starch *vs*. fibre acid detergent in diet.

CONCLUSIONES GENERALES

El uso de combinar urea de lenta liberación y urea grado alimenticio en dietas de finalización para rumiantes puede presentar ventajas sobre la ganancia diaria y/o la eficiencia de la utilización de la energía de la dieta. De acuerdo a los resultados obtenidos, el nivel de respuesta está supeditado a la relación existente de almidón y fibra detergente ácido contenida en la ración. Está relación está estrechamente asociada con la velocidad de fermentación de los carbohidratos y el N de la dieta favoreciendo una mejor sincronía entre ambos nutrimentos. Los resultados obtenidos sobre los flujos de nitrógeno amoniacal a duodeno confirman de la protección de la urea mediante el tratamiento con la cubierta polimérica.

De igual manera, la mayor eficiencia de la producción de proteína microbiana, así como el incremento en las tasas de fermentación de MO promovieron una mejor utilización de la energía de la dieta traduciéndose en mejoras en la eficiencia alimenticia y retención de energía de la misma sin efectos sobre las características de la canal. Estos beneficios se disminuyen a medida que la relación almidón:FDA se aleja (por encima o por debajo) de 4.5.

El costo-beneficio de esta práctica lo determinarán el costo del producto (Optigen), el precio del grano y del forraje que lo sustituye, así como el precio de la carne en canal.