INFLUENCE OF NUTRITION ON METHANE GAS PRODUCTION IN MURCIANO-GRANADINA GOATS

This Thesis has been submitted in accordance with the requirements for the degree of Doctor at the Universitat Politècnica de València.

PhD Thesis
Carla Ibáñez Sanchis

Thesis Supervisor
Dr. Carlos Javier Fernández Martínez

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Ibáñez, C.

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THESIS EVALUATORS

First evaluator
Dr. Fernando Emilio Bacha Baz
Nacoop, S.A.

Second evaluator
Dr. Juan Carlos Gardón Poggi
Universidad Católica de Valencia San Vicente Mártir

Third evaluator
Dr. José Sansano Maestre
Universidad Católica de Valencia San Vicente Mártir

THESIS COMMITTEE

President
Dr. Francisco Juan Espinós Gutiérrez
Universitat Politècnica de València

Secretary
Dr. Fernando Emilio Bacha Baz
Nacoop, S.A.

Vocal
Dr. Antonio Calvo Capilla
Universidad Católica de Valencia San Vicente Mártir
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<td>Acid detergent fiber</td>
</tr>
<tr>
<td>BHBA</td>
<td>β-hydroxybutyrate</td>
</tr>
<tr>
<td>BP</td>
<td>Beet pulp diet</td>
</tr>
<tr>
<td>BRL</td>
<td>Barley diet</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
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<tr>
<td>CB</td>
<td>Carbon balance</td>
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<td>Methane</td>
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<td>Carbon</td>
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<td>Carbon in milk</td>
</tr>
<tr>
<td>C&lt;sub&gt;urine&lt;/sub&gt;</td>
<td>Carbon in urine</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CO₂ₓ</td>
<td>Carbon dioxide production from oxidation</td>
</tr>
<tr>
<td>CORN</td>
<td>Diet corn</td>
</tr>
<tr>
<td>CP</td>
<td>Crude protein</td>
</tr>
<tr>
<td>DEI</td>
<td>Digestible energy intake</td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>DMI</td>
<td>Dry matter intake</td>
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<td>EE</td>
<td>Ether extract</td>
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<td>E&lt;sub&gt;urine&lt;/sub&gt;</td>
<td>Energy retained in urine</td>
</tr>
<tr>
<td>GE</td>
<td>Gross energy</td>
</tr>
<tr>
<td>GEI</td>
<td>Gross energy intake</td>
</tr>
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<td>H₂</td>
<td>Dihydrogen</td>
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<tr>
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<td>Phosphoric acid</td>
</tr>
<tr>
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</tr>
<tr>
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<td>Sulfuric acid</td>
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<tr>
<td>IC</td>
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<td>K&lt;sub&gt;i&lt;/sub&gt;</td>
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</tr>
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<td>Low starch diet</td>
</tr>
<tr>
<td>ME</td>
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</tr>
<tr>
<td>MEI</td>
<td>Metabolizable energy intake</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>N&lt;sub&gt;feces&lt;/sub&gt;</td>
<td>Nitrogen in feces</td>
</tr>
</tbody>
</table>
\( N_{\text{intake}} \) Nitrogen intake
\( N_{\text{milk}} \) Nitrogen in milk
\( N_{\text{urine}} \) Nitrogen in urine
\( \text{NB} \) Nitrogen balance
\( \text{NDF} \) Neutral detergent fiber
\( \text{NEL} \) Net energy for lactation
\( \text{NEFA} \) Non esterified fatty acids
\( \text{NFC} \) Non fibrous carbohydrates
\( \text{NH}_3\text{~N} \) Ammonia nitrogen
\( \text{N}_2\text{O} \) Nitrous oxide
\( \text{O}_2 \) Oxygen
\( \text{OH} \) Hydroxil radical
\( \text{OM} \) Organic matter
\( \text{OMI} \) Organic matter intake
\( \text{OP} \) Orange pulp diet
\( \text{OXCHO} \) Oxidation of carbohydrates
\( \text{OXF} \) Oxidation of fat
\( \text{OXP} \) Oxidation of protein
\( \text{RE} \) Retained energy
\( \text{RE}_{\text{body}} \) Retained energy in body tissues
\( \text{RE}_{\text{fat}} \) Retained energy as fat
\( \text{RE}_{\text{milk}} \) Retained energy as milk
\( \text{RE}_{\text{protein}} \) Retained energy as protein
\( \text{RE}_{\text{total}} \) \( \text{RE}_{\text{protein}} + \text{RE}_{\text{fat}} \)
\( \text{RQ}_{\text{npx}} \) Non protein respiratory quotient from oxidation of nutrients
\( \text{SEM} \) Standard error of the mean
\( \text{SF}_6 \) Sulphur hexafluoride
\( \text{SH} \) Soybean hulls diet
\( \text{T} \) Million tonne
\( \text{TE} \) Tissue energy
\( \text{VFA} \) Volatile fatty acids
\( \text{Ym} \) Methane energy/gross energy intake
Climate change, a resultant effect of greenhouse gas emissions, is a worldwide concern because its continuation is having significant impacts on people, natural resources and economic conditions around the world. The root cause of this recent past and projected climate change is now recognised to be the warming potential of a number of greenhouse gases that, by absorbing terrestrial infrared radiation, raise the temperature of the troposphere and with it, global surface temperatures.

The major greenhouse gases are water vapour, carbon dioxide, methane, nitrous oxide and fluorinated gases. While carbon dioxide receives the most attention as a factor which causes global warming, methane also cause significant radiative forcing. With the relative global warming potential of 20-25 compared with carbon dioxide, methane is one of the most important greenhouse gases.

Methane is only second to carbon dioxide in its contribution to global warming and its emissions are caused by both natural and anthropogenic actions. Human activities such as intensive livestock farming are the primary cause of the increased methane concentrations in the atmosphere, being ruminants the animals which create large amounts of methane via fermentation of feeds in the rumen. During this physiological digestive process, hydrogen is released by some microbes during fermentation of forage and is used by methanogenic Archaea (methanogens) to convert carbon dioxide to methane, which is released through eructation, normal respiration and small quantities as flatus.

Rumen fermentation of cattle contributes the most towards the greenhouse effect through methane emission followed by sheep, goats and buffalos, respectively. Methane emitted from ruminants is not only an environmental threat, it also represents a loss of 2-12% of the ruminant’s gross energy intake, so it is an important issue to improve feed efficiency and increase profitability, too.

Several techniques have been developed to quantify methane emissions from ruminants – indirect calorimetry, sulphur hexafluoride tracer technique and in vitro gas production technique – and some strategies for reduction of methane
Abstract
emissions from the rumen have been described – defaunation treatment, vaccine and dietary composition –.

The initial topics of this research were: design the experiments with goats because there are not many reports about methane emissions in these animals; investigate the influence of dietary composition (carbohydrates) as a strategy for reduction methane emissions from the rumen; and use the indirect calorimetry as method to quantify methane production.

Consequently, three experiments were performed. Murciano-Granadina goats during mid or late lactation were used. Diets were mixed rations that differed in the inclusion of cereal or fibrous by-products. The effect of diet was studied on milk yield, digestibility, rumen parameters, energy partitioning, carbon and nitrogen balance, substrate oxidation and methane productions.

In the first experiment, gas exchange was measured using a face mask which was fixed to the head of the goat by a rubber band; a sample of exhaled gas was stored in a gas collection bag which was connected to an analyzer, and it measured the concentration of O₂, CO₂ and CH₄ from the air. This first experiment replaces corn grain with beet pulp and the amount of methane recovered was 19.6 and 29.7 g/day, respectively.

In the other two experiments, gas exchange was measured by a head box designed for small ruminants where the goat introduced the whole head and a specific software automatically recorded concentrations of O₂, CO₂ and CH₄ from the exhaled air continuously throughout the day. The second experiment involved two diets with high and low level of starch and no differences were found on methane emission (28.5 g/day). The experiment number three replaces ingredient by ingredient like in the experiment number one. Here, barley grain was replaced with orange pulp or soybean hulls and no differences were found, with an average methane production value of 41 g/day. The metabolizable energy intake during the three experiments was 1279 kJ/kg of BW⁰.⁷₅ and day on average, and the efficiency use of metabolizable energy intake for milk production was 0.6.
El cambio climático es una preocupación de ámbito mundial debido a que su perpetuación en el tiempo está teniendo un impacto significativo sobre las personas, los recursos naturales y las condiciones económicas de todo el mundo. La causa fundamental de este pasado reciente y futuro cambio climático es el potencial de calentamiento de una serie de gases de efecto invernadero que, mediante la absorción de la radiación infrarroja terrestre, elevan la temperatura de la troposfera y, con ella, las temperaturas superficiales de la Tierra.

Los principales gases de efecto invernadero son el vapor de agua, el dióxido de carbono, el metano, el óxido nitroso y los gases fluorados. Mientras que el dióxido de carbono recibe la mayor atención como factor que causa el calentamiento global, el metano también causa un forzamiento radiativo significativo. Con una relación potencial de calentamiento global de 20-25 en comparación con el dióxido de carbono, el metano es uno de los gases de efecto invernadero más importantes.

El metano, después del dióxido de carbono, es el gas que más repercusión tiene sobre el calentamiento global y sus emisiones son causadas tanto por acciones naturales como humanas. Actividades antropógicas tales como la ganadería intensiva son la principal causa de aumento de las concentraciones de metano en la atmósfera, siendo los rumiantes los animales que mayores cantidades de metano generan a través de la fermentación de alimentos que se produce en el rumen. Durante este proceso digestivo fisiológico, el hidrógeno es liberado por algunos microorganismos durante la fermentación del forraje y este es utilizado por las arqueobacterias metanogénicas para convertir el dióxido de carbono en metano, el cual es liberado a través de la boca, la nariz o en menor medida vía rectal.

Las emisiones de metano del ganado vacuno son las que principalmente contribuyen al efecto invernadero seguido de las ovejas, las cabras y los búfalos, respectivamente. El metano emitido por los rumiantes no es solo una amenaza medioambiental, sino que además puede representar una pérdida de un 2-12% de la energía bruta ingerida por el animal, por lo que también es una cuestión
importante a tener en cuenta para mejorar la eficiencia de la alimentación y aumentar la rentabilidad.

Se han descrito diferentes técnicas para medir las emisiones de metano de los rumiantes – la calorimetría indirecta, la técnica del marcador con hexafluoruro de azufre y la técnica de producción de gas in vitro – y, además, se han mencionado algunas estrategias para reducir las emisiones de metano – la defaunación, las vacunas y la composición de la dieta –.

Los puntos clave de esta Tesis fueron: diseñar los experimentos con cabras, debido a que no hay muchas investigaciones sobre emisiones de metano en estos animales; estudiar la influencia de la composición de la dieta (hidratos de carbono) como una posible estrategia para la reducción de las emisiones de metano del rumen; y utilizar la calorimetría indirecta como método para cuantificar la producción de metano.

Se realizaron tres experimentos. Se utilizaron cabras de la raza Murciano-Granadina en mitad o final de la lactación. Las dietas eran raciones mixtas que diferían en la inclusión de cereal o subproductos fibrosos. El efecto de la dieta se estudió en la producción de leche, la digestibilidad, los parámetros del rumen, la partición de energía, el balance de carbono y nitrógeno, la oxidación de nutrientes y las producciones de metano.

En el primer experimento, el intercambio de gases se midió utilizando una mascarilla que se fijó a la cabeza de la cabra con una goma; se almacenó una muestra de gas exhalado en una bolsa de recogida de gas que estaba conectada a un analizador, y se midió la concentración de O₂, CO₂ y CH₄ del aire. En este primer experimento se reemplazó el grano de maíz con pulpa de remolacha y la cantidad de metano recuperado fue del 19,6 y 29,7 g/día, respectivamente.

En los otros dos experimentos, el intercambio de gases se midió mediante una urna o cajón diseñado para pequeños rumiantes, donde la cabra introducía toda la cabeza y un programa informático grababa automáticamente las concentraciones de O₂, CO₂ y CH₄ del aire exhalado de
forma continua a lo largo del día. El segundo experimento consistió en dos dietas con alto y bajo nivel de almidón y no se encontraron diferencias en la emisión de metano (28,5 g/día). En el tercer experimento se sustituyó ingrediente por ingrediente como en el experimento número uno. El grano de cebada se sustituyó por pulpa de naranja o cascarilla de soja y tampoco se encontraron diferencias, con un valor promedio de la producción de metano de 41 g/día. La energía metabolizable ingerida durante los tres experimentos fue de 1279 kJ/kg de peso metabólico (PV^{0.75}) y día de promedio, y la eficiencia de utilización de la energía metabolizable ingerida para la producción de leche fue de 0,6.
El canvi climàtic és una preocupació d'àmbit mundial ja que la seua perpetuació en el temps està tenint un impacte significatiu sobre les persones, els recursos naturals i les condicions econòmiques de tot el món. La causa fonamental d'aquest passat recent i futur canvi climàtic és el potencial d'escalfament d'una sèrie de gasos d'efecte hivernacle que, mitjançant l'absorció de la radiació infraroja terrestre, elevan la temperatura de la troposfera i, amb ella, les temperatures superficials de la Terra.

Els principals gasos d'efecte hivernacle són el vapor d'aigua, el diòxid de carboni, el metà, l'òxid nitrós i els gasos fluorats. Mentre que el diòxid de carboni rep la major atenció com a factor que causa l'escalfament global, el metà també causa un forçament radiatiu significatiu. Amb una relació potencial d'escalfament global de 20-25 en comparació amb el diòxid de carboni, el metà és un dels gasos d'efecte hivernacle més importants.

El metà, després del diòxid de carboni, és el gas que més repercussió té sobre l'escalfament global i les seues emissions són causades tant per accions naturals com humanes. Activitats antropogèniques com ara la ramaderia intensiva són la principal causa d'augment de les concentracions de metà a l'atmosfera, sent els remugants els animals que més quantitats de metà generen a través de la fermentació d'aliments que es produeix al rumen. Durant aquest procés digestiu fisiològic, l'hidrogen és aliberat per alguns microorganismes durant la fermentació del farratge i aquest és utilitzat per arqueobacteris metanogènics per convertir el diòxid de carboni en metà, el qual és aliberat a través de la boca, el nas o en menor mesura via rectal.

Les emissions de metà dels bovins són les que principalment contribueixen a l'efecte hivernacle seguit de les ovelles, les cabres i els búfals, respectivament. El metà emès pels remugants no és només una amenaça mediambiental, sinó que a més pot representar una pèrdua d'un 2-12% de l'energia bruta ingerida per l'animal, de manera que també és una qüestió important a tenir en compte per millorar l'eficiència de l'alimentació i augmentar la rendibilitat.

S'han descrit diferents tècniques per mesurar les emissions de metà dels remugants – la calorimetria indirecta, la tècnica del marcador amb hexafluorur
Resum

de sofre i la tècnica de producció de gas in vitro – i, a més, s’han esmentat algunes estratègies per reduir les emissions de metà – la defaunació, les vacunes i la composició de la dieta –.

Els punts clau d’aquesta Tesi van ser: dissenyar els experiments amb cabres, pel fet que no hi ha moltes investigacions sobre emissions de metà en aquests animals; estudiar la influència de la composició de la dieta (hidrats de carboni) com una possible estratègia per a la reducció de les emissions de metà del rumen; i utilitzar la calorimetria indirecta com a mètode per quantificar la producció de metà.

Es van realitzar tres experiments. S’utilitzaren cabres de la raça Murciano-Granadina a la meitat o final de la lactació. Les dietes eren racions mixtes que diferien en la inclusió de cereal o subproductes fibrosos. L’efecte de la dieta es va estudiar en la producció de llet, la digestibilitat, els paràmetres del rumen, la partició d’energia, el balanç de carboni i nitrogen, l’oxidació de nutrients i les produccions de metà.

En el primer experiment, l’intercanvi de gasos es va mesurar utilitzant una màscara que es va fixar al cap de la cabra amb una goma; es va emmagatzemar una mostra de gas exhalat en una bossa de recollida de gas que estava connectada a un analitzador, i es va mesurar la concentració d’O₂, CO₂ i CH₄ de l’aire. En aquest primer experiment es va reemplaçar el gra de blat de moro amb polpa de remolatxa i la quantitat de metà recuperat va ser del 19,6 i 29,7 g/dia, respectivament.

En els altres dos experiments, l’intercanvi de gasos es va mesurar mitjançant una urna o calaix dissenyat per a petits remugants, on la cabra introdueix tot el cap i un programa informàtic gravava automàticament les concentracions d’O₂, CO₂ i CH₄ de l’aire exhalat de forma contínua al llarg del dia. El segon experiment va consistir en dues dietes amb alt i baix nivell de midó i no es van trobar diferències en l’emissió de metà (28,5 g/dia). En el tercer experiment es va substituir ingredient per ingredient com en l’experiment número u. El gra d’ordi es va substituir per polpa de taronja o pellofa de soja i tampoc es van trobar diferències, amb un valor mitjà de la producció de metà de 41 g/dia. L’energia
metabolitzable ingerida durant els tres experiments va ser de 1279 kJ/kg de pes metabòlic (PV^{0.75}) i dia de mitjana, i l'eficiència d'utilització de l'energia metabolitzable ingerida per a la producció de llet va ser de 0,6.
Greenhouse gases

Increases in the amount of greenhouse gases have become an important worldwide topic due to their effects on global warming and consequently on climate change. The effects of these emissions on the ecological and socioeconomic vulnerability have already been noticed and will continue to grow regionally and globally in the years to come.

Greenhouse gases are a group of compounds that are able to trap heat in the atmosphere. These gases absorb and emit radiation within the thermal infrared range and are the fundamental cause of the greenhouse effect. Greenhouse gases greatly affect the temperature of the Earth, keeping the Earth’s surface warmer than it would be if they were not present.

The principal forcing greenhouse gases are carbon dioxide (CO$_2$), methane (CH$_4$), nitrous oxide (N$_2$O) and fluorinated gases (hydrofluorocarbons, perfluorocarbons and sulphur hexafluoride), and the main feedback greenhouse gas is water vapor.

Forcing greenhouse gases take many years to leave the atmosphere. Carbon dioxide, methane, nitrous oxide and the fluorinated gases are all well-mixed gases in the atmosphere. They do not react to changes in either temperature or air pressure and thus do not get removed easily like water that condenses to become rain or snow. Their long atmospheric lifetimes allows them to have a lasting effect on global warming and climate change. Water vapor on the other hand has a residence time of a few days. It is a highly active component of the climate system that responds rapidly to changes in conditions by either condensing into rain or snow, or evaporating to return to the atmosphere. Thus the impact of the greenhouse effect is primarily circulated through water vapor, and it acts as a fast feedback, accentuating the warming provided by the forcing greenhouse gases (O’Mara, 2011).

Forcing greenhouse gases are the key gases within the Earth’s atmosphere that sustain the greenhouse effect and control its strength. Carbon dioxide is the most important one, being responsible for over 70 per cent of the global
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greenhouse gas emissions. Methane is the second most prevalent greenhouse gas emitted in the Earth, which represents 16 per cent of the global greenhouse gas emissions. Nitrous oxide and fluorinated gases are responsible for 10 per cent of the total emissions (Figure 1).

**Figure 1:**

![Global greenhouse gas emissions](image)


While carbon dioxide is typically painted as the “bad boy” of greenhouse gases, methane is roughly more potent as a heat-trapping gas. Although methane’s lifetime in the atmosphere is much shorter than carbon dioxide (12 vs. 200 years), methane can trap 20 to 25 times more heat than carbon dioxide and it is removed when it reacts with the hydroxyl (OH) radical to form carbon dioxide. As happens with carbon dioxide, reducing methane levels is definitely a goal to pursue because reductions realized today can produce important near-term progress toward climate change mitigation (Greenhouse gas emissions. United States Environmental Protection Agency, 2015).
Methane

There are both natural and human sources of methane emissions. The main natural sources include wetlands, termites and the oceans and create 36% of methane emissions. Important human sources come from landfills, livestock farming, as well as the production, transportation and use of fossil fuels. This human-related sources create the majority of methane emissions, accounting for 64% of the total.

Methane levels have more than doubled over the last 150 years because of human activities like fossil fuel use and intensive farming. Before the Industrial Revolution, the atmospheric concentration of methane was maintained in a safe range by natural sinks. But for a long time new human activities have been creating methane emissions much more rapidly than the Earth can remove them, increasing global methane levels.

Human activities such as fossil fuel production and intensive livestock farming are the primary cause of the increased methane concentrations in the atmosphere. Together these two sources are responsible for 60% of all human methane emissions. Other sources include landfills and waste (16%), biomass burning (11%), rice agriculture (9%) as well as biofuels (4%) (Figure 2).

An important source of methane emissions is from rumen fermentation in farm animals. This is responsible for 27% of human methane emissions. Animals like cows, sheep and goats create large amounts of methane during their normal digestion process and because humans raise these animals for food, their emissions are considered human-related. The meat, milk that we eat, drink everyday contribute significantly to total methane emissions because of this. Domesticated ruminants produce as much as 86 million tonnes (T) of methane per year. Approximately 18.9 T are from dairy cattle, 55.9 T are from beef cattle, and 9.5 T are from sheep and goats; this last value includes emissions from both species due to there are not too much studies about methane emissions in small ruminants, specially in goats. Data from Johnson and Ward (1996), estimates the global yearly methane contribution of buffalo to be 6.2-8.1 T, 0.9-1.1 T from...
camels, and methane production within the hindgut of pigs and horses to be approximately 0.9-1.0 T and 1.7 T, respectively.

**Figure 2:**

![Human sources of methane](image)

**Source:** Bousquet et al. (2006). Contribution of anthropogenic and natural sources to atmospheric methane variability.

Methane emission from ruminants is not only a worldwide environmental issue, also represents a loss of 2-12% of the ruminant’s gross energy intake depending upon the diet, so it is an important topic to improve feed efficiency and increase profitability, too (Knapp et al., 2014).

**Rumen fermentation**

Rumen fermentation is the anaerobic fermentation of polysaccharides and other feed components in the rumen of animals. Methane is produced as a waste product of this fermentation process. Although methane production can also occur in the lower gastrointestinal tract, as in non ruminants mammals, 89%
of methane emitted from ruminants is produced in the rumen and exhaled through the mouth and nose, the rest is released via rectal.

The rumen forms a huge fermentation vat, containing billions of microorganisms which anaerobically break down the ingested material before it is enzymatically digested by the ruminant. Nutritional components such as carbohydrates, proteins and lipids in feedstuffs are degraded by rumen microorganisms and are converted into microbial cells, which include proteins and carbohydrates, and volatile fatty acids (VFA) and gases. Because hydrogen derives mainly from carbohydrates and is supplied for methane production in the rumen, carbohydrate degradation is often the focus of efforts to abate methane production from livestock rumens (Mitsumori and Sun, 2008).

Various carbohydrates contained in feedstuffs are degraded by rumen fermentation, which is carried out by a consortium of microorganisms, the rumen microbial ecosystem. Although rumen bacteria are abundant and it is rumen bacteria that mainly support rumen fermentation, rumen protozoa and rumen fungi, which are anaerobic eukaryotes, also contribute to rumen fermentation. Fermentation pathways relating to carbohydrate utilization in the rumen have been intensively investigated; the results of such studies have shown that the major products of rumen fermentation are VFA, CO₂ and CH₄ (Russell and Wallace, 1997). Predominant VFA from the rumen include acetate, propionate and butyrate. The stoichiometry of fermentation of hexose to the three main VFA according to Orskov and Ryle (1990) is shown below:

Acetic acid:

\[ C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \]

Propionic acid:

\[ C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3-C\_2\_COOH + 2H_2O \]

Butyric acid:

\[ C_6H_{12}O_6 \rightarrow CH_3-C\_2\_CH_2-COOH + 2CO_2 + 2H_2 \]

\[ 4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \]
While rumen fermentation is generally performed by the rumen microbial ecosystem, individual rumen microorganisms degrade specific substrates for their growth. The rumen microorganisms eventually release into the rumen their final products of metabolism, or fermentation products, some of which are utilized by other microorganisms. For example, *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* can degrade cellulose and are thus referred to as cellulytic bacteria. *F. succinogenes* and *R. flavefaciens* produce acetate and succinate as major fermentation products; *R. albus* produces only acetate. Succinate, produced by *Prevotella ruminicola*, *Ruminobacter amylophilus*, *F. succinogenes*, *R. flavefaciens*, *Succinivibrio dextrinosolvens*, *Succinomonas amylolytica* and other bacteria, is continuously converted to propionate and CO$_2$ by *Selenomonas ruminantium*, for which succinate is a propionate-generating pathway intermediate, and *Veillonella alcalescens* and *Succinilasticum ruminis* and others, which decarboxylate succinate to produce propionate (Wolin et al., 1997). Therefore, rumen fermentation is properly expressed as the total of metabolisms of individual microorganisms inhabiting the rumen.

The carbohydrate-fermenting bacteria and protozoa in the rumen produce CO$_2$, H$_2$ and VFA. It is known that CO$_2$ and H$_2$ are major precursors of CH$_4$; formate is also a precursor of CH$_4$. Methane production from formate is estimated to comprise approximately 15-20% of the total methane production in the rumen (Hungate et al., 1970; Asanuma et al., 1999). The precursors for methane production are converted into CH$_4$ by methane-producing Archaea, methanogens that appeared on Earth some 3.5×10$^9$ years ago (Ueno et al., 2006). *Methanobrevibacter ruminantium*, *Methanomicrobium mobile*, *Methanosarcina mazei*, *Methanosarcina barkeri* and *Methanobacterium formicicum* have been isolated from the rumen by cultivation (Mitsumori et al., 2002). Biochemical studies of culturable methanogens have shown that *M. ruminantium*, *M. formicicum* and *M. mobile* utilize H$_2$/CO$_2$ and formate to produce methane. On the other hand, *M. mazei* synthesizes methane from acetate, methanol and methylamines. *M. barkeri* utilizes H$_2$/CO$_2$, acetate, methanol and methylamines for methane synthesis (Jarvis et al., 2000). It is...
assumed that methyl coenzyme-M reductase is common in the methanogens, because the final step of methane production by the methanogens is catalyzed by this methyl coenzyme-M reductase (Ermler et al., 1997).

**Methods to measure methane emissions**

Different methods have been developed with the purpose of quantify methane emissions from ruminants. The best-known methods are indirect calorimetry, sulphur hexafluoride tracer technique and in vitro gas production technique.

**Indirect calorimetry**

Measurements of ruminant CH\textsubscript{4} emissions have been undertaken using indirect calorimetry techniques that calculated energy expenditure by the consumption of oxygen and expiration of CO\textsubscript{2}. In contrast, direct calorimetry determines the energy expenditure of an animal by directly measuring heat production (Blaxter, 1989).

Indirect calorimetry techniques have been used effectively to collect most of the available information concerning CH\textsubscript{4} emissions in livestock. The predominant use of calorimeters has been to measure gaseous exchange as part of energy balance measurements, CH\textsubscript{4} loss being a necessary part of this procedure. There are various designs of calorimeters, they can either be closed or open circuit calorimeters, with the open circuit systems being the most common (Pinares-Patiño and Clark, 2008). Open circuit systems involve the continuous flow of outside air circulating around the animal’s head, mouth and nose and well mixed inside, air is collected. The difference between CH\textsubscript{4} in air entering and exiting, once adjusted for flow rate, allows the determination of CH\textsubscript{4} emissions. In contrast, in closed circuit systems air conditioning and CO\textsubscript{2} absorbents maintain the circulation of the air, the quality (humidity and temperature) and CO\textsubscript{2} concentration. Thus, the closed circuit must not have any air leakage to ensure the accurate measurement of gas emissions. A distinct disadvantage of the closed circuit respiration technique is that despite the
conditioning of the air gaseous emissions can only be measured for very short periods, as animals are at risk of running out of oxygen.

Indirect calorimetry techniques such as whole animal chambers, head boxes or ventilated hoods and face masks have recently become the preferred technique to measure CH\textsubscript{4} emissions from ruminants.

- **Respiration chamber or whole animal chambers:**

The animal is placed in open-circuit respiration chamber for a period of several days, the inputs (feed, oxygen, CO\textsubscript{2}) and outputs (excretion, oxygen, CO\textsubscript{2} and CH\textsubscript{4}) were measured from the chamber (Miller and Koes, 1988). The chamber should be well sealed and capable of a slight negative pressure. This ensures that all leaks will be inward and not result in a net loss of CH\textsubscript{4}. Air conditioning, dehumidification, feeders, waterers and a method by which feces and urine could be removed are necessary in order to create a comfortable environment within the chamber. Animal movement and normal behaviour should be provided for as much as possible however, some degree of restraint is necessary within the chamber.

**Advantages**

The ability to make accurate measurements of emissions including CH\textsubscript{4} from ruminal and hindgut fermentations.

**Disadvantages**

While this technique is satisfactory for measuring CH\textsubscript{4} emission from dried diets, there are difficulties in deriving values that are applicable to the grazing ruminant.

a) Grazing ruminant select their diet, maximum intakes in a chamber are considerably lower than in grazing animals, fresh pasture continue to respire in the chamber.

b) The restriction of the animal movement
c) The expenses associated with the construction and maintenance of the chambers.

- Head boxes or ventilated hoods:

A ventilated hood could also be used to quantify CH$_4$ emissions using the same principles (Takahashi et al., 1999). This technique involves the use of an airtight box that surrounds the animal’s head. A sleeve or drape could be placed around the neck of the animal to minimize air leakage. The box must be big enough to allow the animal to move its head in an unrestricted manner and allows access to feed and water.

Advantages

The primary advantage of this technique is the relatively lower cost of the ventilated hood system as compared to a whole animal chamber.

Disadvantages

a) As with the chamber, use of a hood also requires a restrained and trained animal

b) The inability to measure all the hindgut CH$_4$

- Face masks:

Face masks may also be used to quantify CH$_4$ production (Liang et al., 1989). The principle behind the use of the facemask is the same as that of the chamber and hood.

Advantages

The primary advantages of this method are the simplicity and lower cost. They can also be used to collect the expired gas from the grazing animals periodically and estimate CH$_4$ production.

Disadvantages

The facemask, compared with chamber methods, underestimates heat production and likely CH$_4$ as well by an average 9% (Liang et al., 1989). It requires
animal cooperation and eliminates its ability to eat and drink. Because of the normal daily variation in emissions meaningful CH\(_4\) emission measurements is difficult and hence short term measurements might lead to erroneous results (Johnson and Johnson, 1995).

**Sulphur hexafluoride tracer technique**

The SF\(_6\) technique was first developed by Johnson et al. (1994) and involves the placement of a permeation tube charged with SF\(_6\) liquid into the rumen of the animal. Sulphur hexafluoride is released from the permeation tube as a gas via the Teflon® membrane. The rate of SF\(_6\) gas release is determined by gravimetric weighing for at least two months prior to placement into the rumen, whilst permeation tubes are kept at 39°C (Lassey et al., 1997; 2001). Breath samples from the animal contain both SF\(_6\) and CH\(_4\) gases, and are collected continuously over a 24 hour period via equipment mounted on the animal’s head. Samples are then stored in an evacuated canister and later analyzed by gas chromatography (Lassey et al., 1997; 2001). The methane emission is calculated from the release rate of SF\(_6\) as described the following equation:

\[
F_{CH4} = F_{tracer} \times \left[ \frac{(C_{\text{measured CH4}} - C_{atm CH4})}{(C_{\text{measured tracer}} - C_{atm tracer})} \right]
\]

where \(F_{CH4}\) is the total production of CH\(_4\), \(F_{tracer}\) is the total production or release of SF\(_6\), \(C_{\text{measured CH4}}\) and \(C_{\text{measured tracer}}\) are the measured concentrations of CH\(_4\) and SF\(_6\) in the experimental canister, while \(C_{atm CH4}\) and \(C_{atm tracer}\) are the concentrations of CH\(_4\) and SF\(_6\) in atmosferic air, measured with the same analyzer and in the same unit (ppm).

Relative to indirect calorimetry, the SF\(_6\) method is less expensive than chambers and measurements can be made from large numbers of grazing animals, which is important for pastoral systems. However, this latter advantage is offset by the difficulty in determining the feed intake of grazing animals. The lack of accuracy in estimating dry matter intake (DMI) from grazing animals could mean that any small decrease in intake, due to the intensive handling of animals or to the wearing of breath collection equipment, will not detected,
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thereby diminishing the precision of calculated CH₄ yield (CH₄ expressed per unit of DMI).

The accuracy of the SF₆ technique to estimate CH₄ emissions is reliant on several assumptions, which are: that the SF₆ gas simulates the emissions of CH₄ and is uniformly mixed; dilution rates of SF₆ and CH₄ gases are identical; SF₆ is inert; the release rate of gas from the permeation tube follows a constant linear pattern; and there are no interactions between rumen contents and SF₆ gas (Johnson et al., 1994; Ulyatt et al., 1999; Boadi et al., 2002; Vlaming et al., 2007; Pinares-Patiño and Clark, 2008). In addition, it is critical that the release rate of SF₆ from the permeation tubes in the rumen is accurately determined.

In vitro gas production technique

The in vitro gas production technique has been used to simulate ruminal fermentation of feed and feedstuffs for decades. With the increasing interest in greenhouse gas emissions from agriculture in recent years, the traditional in vitro gas production techniques have been modified to include measurement of methane production.

The basic principle of this technique is to ferment feed under controlled laboratory conditions employing natural rumen microbes. Feedstuffs, e.g., subjected to different treatments, are incubated at 39 °C with a mixture of rumen fluid, buffer and minerals for a certain time period, typically 24, 48, 72, 96 or 144 h. The amount of total gas produced during incubation is measured and its composition analyzed, to obtain data on the in vitro production of methane. At the same time it is possible to determine in vitro degradation of the feedstuffs, making it possible to determine whether a reduction in methane production is at the cost of total feed degradation (Storm et al., 2012).

Strategies for reduction of methane emissions from the rumen

Reducing methane emissions is a powerful way to take action on climate change. Some of the following strategies could be used to reduce methane from the rumen.
Defaunation treatment

Defaunation, which is the removal of protozoa from the rumen, has been used to investigate the role of protozoa in rumen function, and also to study the effect on methane production. Rumen protozoa share a symbiotic relationship with methanogens, participating in interspecies hydrogen transfer, which provides methanogens with the hydrogen they require to reduce carbon dioxide to methane (Machmüller et al., 2003a). It has been estimated that the methanogens associated with the ciliate protozoa, both intracellularly and extracellularly, are responsible for 9 to 37% of the methane production in the rumen (Finlay et al., 1994; Newbold et al., 1995). For this reason, treatments that decrease the protozoal population of the rumen, may also decrease the protozoa-associated methanogen population and therefore, decrease the methane production within the rumen. Treatments that have been used include copper sulphate, acids, surface-active chemicals, triazine, lipids, tannins, ionophores, and saponins (Hobson and Stewart, 1997). It has been suggested that the effect of defaunation on methane output is diet dependent. Hegarty (1999) found that defaunation reduced methane output 13%, but the magnitude of reduction varied with diet. The greatest reduction in methane production with defaunation was measured on a high-concentrate diet, likely because protozoa are the predominant source of hydrogen for methanogenesis on starch-based diets.

Vaccine

Another methane reduction strategy that is being investigated is the development of a vaccine that would stimulate the ruminant’s immune system to produce antibodies against methane-producing methanogens (Wright et al., 2004).

Dietary composition

The dietary proportions of cellulose, hemicellulose and soluble carbohydrates influence microbial fermentation pathways and consequently, production of methane. The components of the diet fed, especially type of
carbohydrate, are important for methane production as they are able to influence the ruminal pH and subsequently alter the microbiota present. The digestibility of cellulose and hemicellulose are strongly related to methane production, more so than soluble carbohydrate. In a study by Holter and Young (1992), a positive relationship was found between digestibility of hemicellulose and methane output in forage fed non-lactating cows. A negative relationship was found between digestibility of cellulose and methane output. Sauvant and Giger-Reverdin (2007) found the relationship between methane production and proportion of concentrate in the diet to be curvilinear, with methane losses of 6-7% of gross energy (GE) being constant at 30-40% concentrate levels in the diet and then decreasing to 2-3% of GE with a concentrate proportion of 80–90%. The starch component of the diet is also known to promote propionate formation, through a shift to amylolytic bacteria, and a reduction in ruminal pH, leading to a decrease in methanogenesis (Van Kessel and Russell, 1996). Johnson and Johnson (1995) stated that the digestion of cell wall fiber increases methane production, by increasing the amount of acetate produced in relation to propionate. The increase in methane output is due to the fermentation of acetate, which provides a methyl group for methanogenesis (Ferry, 1992). Grinding forage feed before it is ingested by the cows also seems to decrease the production of methane, presumably by increasing the rate of digestion and flow through the gastrointestinal tract, thus limiting the time available for methane to be produced within the rumen (Johnson and Johnson, 1995).

It is important to note that increasing the amount of rapidly fermentable carbohydrates in a diet can increase the rate of passage from the rumen, as well as lower the ruminal pH. Increased passage rates can shift methanogenesis to the hind gut, as well as to the manure, possibly off setting any reductions in ruminal methane outputs (Hindrichsen et al., 2006). Further, the ruminal digestion of rapidly fermentable carbohydrates can increase the production of VFA. If VFA production is greater than absorption, the pH in the rumen will drop, leading to subacute ruminal acidosis and disruption of the rumen microbiota (Plaizier et al., 2008).
Lipids:  
Lipids, such as fatty acids and oils, are options for feed supplementation that have been investigated both in vitro and in vivo for their effects on methanogenesis. Increased lipid content in the feed is thought to decrease methanogenesis through inhibition of protozoa, increased production of propionic acid, and by “biohydrogenation of unsaturated fatty acids”. Unsaturated fatty acids may be used as hydrogen acceptors as an alternative to the reduction of carbon dioxide (Johnson and Johnson, 1995). Also, fatty acids are thought to inhibit methanogens directly through binding to the cell membrane and interrupting membrane transport (Dohme et al., 2001). Interestingly, Kong et al. (2010) detected Archaea using fluorescence in situ hybridization in the rumen of dairy cows supplemented with flaxseed, and did not find any obvious differences in the proportion of Archaea present with flaxseed addition. The authors stated that it was possible fatty acid supplementation was affecting activity instead of quantity of methanogens.

A meta-analysis of methane output with lipid supplementation in lactating dairy cows found a 2.2% decrease in methane per 1% of supplemented lipid in the diet (Eugène et al., 2008). In cattle and sheep, Beauchemin et al. (2008) found an association of 5.6% methane reduction per percentage unit of lipid added to the diet. There are many factors that may account for varying effects of lipids on methane abatement, such as the ruminant species, experimental diet, and the type of lipid used.

Fatty Acids:
A number of fatty acids have been investigated in vivo for methane suppressing effect. Myristic acid was found to reduce methane by 22% in sheep fed a forage-based diet and 58% in a concentrate-based diet when 50 mg/kg DM was used (Machmüller et al., 2003b). Odongo et al. (2007) measured a 36% methane reduction in dairy cattle fed a total mixed ration with 5% myristic acid supplementation on a dry matter (DM) basis. In vitro studies have found fatty acids used in combination have the greatest suppression of methanogenesis due to a synergistic effect. Therefore, it is likely that oil supplementation would
provide a more dramatic depression of methane production than individual fatty acids (Soliva et al., 2004).

- Oils:

Oils extracted from plant sources usually contain a favorable amount of medium- to long-chain fatty acids (McGinn et al., 2004). Refined soy oil fed to beef bulls at 6% inclusion reduced methane production by 39% in terms of litres per day (Jordan et al., 2006). Sunflower oil is more often studied and has resulted in an 11.5-22.0% reduction in methanogenesis (Beauchemin et al., 2007). Sunflower oil has also been combined with linseed oil at a ratio of 1:3 and fed to sheep on a pasturebased diet in a dose-response trial, but at 1.2-5% oil inclusion on a dry matter basis, there was no significant reduction in methanogenesis (Cosgrove et al., 2008). Linseed oil supplemented at a level of 5% of DM to lactating dairy cows resulted in a 55.8% reduction in grams of methane per day (Martin et al., 2008). Coconut oil is the most popular oil for methane abatement experiments and has been found to induce significant reductions in methanogenesis, although the extent of the reduction varies from 13-73%, depending on the inclusion level, diet and ruminant species used (Machmüller et al., 1999; 2000). Since coconut oil has a ratio of lauric to myristic acid of 2.6:1.0, similar to the effective ratios for methane abatement of 4:1, 3:2, and 2.5:2.5 found in vitro by Soliva et al. (2004), it is expected that this oil would provide significant reductions in methanogenesis in vivo. Palm kernel oil has a ratio of lauric to myristic acid of 3:1, suggesting a greater efficacy for methane abatement compared to coconut oil, but to our knowledge, there are currently no published reports of palm kernel oil supplementation in vivo. In an in vitro study by Dohme et al. (2000), coconut oil reduced methane by 21% while palm kernel oil reduced methane by 34%, providing more evidence that palm kernel oil may be more efficacious. However, it is important to note, that in vivo studies involving oil supplementation are often accompanied by a reduction in dry matter intake, which can also result in reduced methane production (Machmüller et al., 2000).
- Other Lipid Sources:

Other lipid sources, such as tallow and seeds, have also been investigated for methane suppressing effects. Beauchemin et al. (2007) supplemented heifers with 34 g of tallow per kg DM and found an 11% reduction in g of methane per kg DMI. Jordan et al. (2006) supplemented beef bulls with whole soybean at an inclusion level of 27% DM and, despite palatability issues resulting in up to 60% refusal, found a 25% reduction of liters methane per day. Beauchemin et al. (2007; 2009) conducted two experiments using sunflower seed supplementation with heifers and dairy cows and found a 23% and 10.4% reduction in methanogenesis, respectively. Beauchemin et al. (2009) also supplemented dairy cows with flaxseed and canola seed at 3.3% (DM basis) and reductions in methane were found to be 17.8% and 16.0%, respectively, as g/kg DMI. Machmüller et al. (2000) found reductions in methane on a kg live weight basis from supplementation of rapeseed, sunflower seed, and linseed of 19%, 27%, and 10%, respectively, in growing lambs. Finally, Grainger et al. (2010) fed 2.61 kg (DM basis) of whole cottonseeds to lactating cows and found the average reduction in methane over the twelve-week experiment was 2.9% per 1% fat addition, with 1.5% reduction at week three and 4.4% at week twelve.

No matter what the lipid form used for supplementation, it is important to consider the ruminant species and the diet being examined, as methane reductions can vary depending on the feed components present. Further, lipid inclusion can affect palatability, intake, animal performance, and milk components, all of which can have implications for practical on-farm use (Odongo et al., 2007; Jordan et al., 2006). Finally, the majority of in vivo experiments conducted to investigate lipids as methane abatement strategies are short-term, making it nearly impossible to draw conclusions about long-term repressive effects. Therefore, long-term supplementation experiments need to be conducted to thoroughly gauge the efficacy of lipid supplementation as an abatement strategy.
- Plant Compounds:

The three main plant compounds effective at reducing methane emissions *in vitro* are condensed tannins, saponins, and essential oils. *In vivo*, the efficacy of these compounds varies in terms of methane abatement.

Condensed tannins are thought to directly inhibit methanogens, as well as indirectly limit methanogenesis through a reduction in hydrogen availability (Tavendale et al., 2005). Condensed tannin-containing *Lespedeza cuneata* was fed to goats *ad libitum* and found to reduce methane 57% in terms of g/kg DMI, compared to goats fed a mixture of *Digitaria ischaemum* and *Festuca arundinacea* (Puchala et al., 2005). Sheep consuming 41 g of tannin-containing *Acacia mearnsii* per kg DM were found to have a 13% reduction in methanogenesis (Carulla et al., 2005). Tannin-containing *Callinada calothyrsus* and *Fleminga macrophylla* also reduced methane 24% in lambs (Tiemann et al., 2008), but an extract of condensed tannin from *Schinopsis quebracho colorado* (Beauchemin et al., 2007) and tannin-containing sorghum silage (Oliveira et al., 2007) fed to cattle did not suppress methanogenesis.

Saponins have been shown *in vitro* to inhibit protozoa, as well as limit hydrogen availability for methanogenesis (Guo et al., 2008). A recent study by Holtshausen et al. (2009) supplemented cows with whole-plant *Yucca schidigera* powder at 10 g/kg DM or whole-plant *Quillaja saponaria* powder at 10 g/kg DM, both of which contain saponin. The authors stated that previous studies *in vitro* had found reductions in methane at higher inclusion levels (15 g/kg DM and greater), but these high levels were avoided *in vivo* in order to minimize effects on digestibility (Guo et al., 2008). No effect of the plant supplementation was found *in vivo* and the authors concluded that the *in vitro* reductions in methane were likely due to reduced feed digestion and fermentation (Holtshausen et al., 2009). This makes *in vivo* supplementation difficult because higher feeding levels may be required to measure reductions in methane output, but these reductions would be at a cost to feed-digestibility.

Essential oils have antimicrobial activities that act in a similar way to monensin by inhibiting gram-positive bacteria (Burt, 2004; Calsamiglia et al., 2007). In this
way, essential oils can reduce the amount of available hydrogen for methanogenesis. Few in vivo studies have been conducted, but one study by Beauchemin and McGinn (2006) where heifers were fed 1 g/d of essential oil and spice extract found no effect on methane output and a negative effect on feed digestibility.

Clearly, more research in necessary in vivo with essential oils, as well as condensed tannins and saponins, to determine the optimal dosage where methanogenesis is reduced without side effects on digestibility. Also, long-term studies are required to determine whether the microbes are able to adapt to supplementation and resume methanogenesis at baseline levels. Finally, it is important to study whether any residues of supplementation appear in milk or meat to make this a viable option for methane abatement in production animals (Calsamiglia et al., 2007).

- **Organic Acids:**

  *In vivo* effects of organic acid supplementation on methane abatement are variable. Wood et al. (2009) supplemented 100 g/kg fumaric acid in the free or encapsulated form to growing lambs and found a 62% and 76% reduction in methane output, respectively. Fumaric acid was also fed to growing beef cattle at 175 g/d, steers at 80 g/d, and wethers at 4-10 g/100 g (dry matter [DM] basis), but was not found to significantly reduce methane emissions, although suppression of DMI was found at higher inclusion levels (McGinn et al., 2004; Molano et al., 2008). Beef heifers were supplemented with 3.75% and 7.5% malic acid on a DM basis and methane output reductions of 3% and 9% as g/kg DMI were measured, respectively (Foley et al., 2009). The authors stated that the effect of organic acid supplementation on methane abatement appears to be influenced by diet, with greater abatement when high-concentrate diets are fed. This is due to a greater effect on the acetate-to-propionate ratio in the rumen, in addition to its ability to act as a hydrogen sink (Foley et al., 2009). Based on the *in vivo* studies presented here, it appears that organic acids may provide beneficial effects in terms of methane abatement, but further *in vivo* experiments need to be conducted to determine the optimal conditions for use. Additionally,
long-term supplementation studies need to be conducted to confirm that any
benefits observed are lasting.

Carro and Ranilla (2003) suggested that malate has a beneficial effect on in
vitro rumen fermentation of cereal grains by increasing volatile fatty acid
concentrations (acetate, propionate and butyrate) and final pH, and by
decreasing lactate concentrations and CH$_4$ production. If these effects are
confirmed in vivo, malate would provide an effective alternative to reduce CH$_4$
emissions.

**Assumptions**

Global warming caused by increasing atmospheric concentrations of
greenhouse gases is a major worldwide environmental, economic and social
threat. The primary concern for livestock production is the methane generated
by ruminant livestock during the normal process of feed digestion. It is well
documented that cows and sheep contribute to this problem, however there
are not many reports about goats; that is why in this research we want to use
goats to develop all the experiments.

In this thesis, we investigate in Murciano-Granadina goats (Capra aegagrus
hircus) the influence of dietary composition as a strategy for reduction methane
emissions from the rumen and use the indirect calorimetry as method to measure
methane production.

**Objectives**

1. To study the effect of substituting corn with beet pulp in the diet of
lactating goats on milk yield, energy partitioning, substrate oxidation and
methane emissions (Experiment 1).

2. To compare the effect of fed two mixed diets to dairy goats differing in the
type of carbohydrate (starch vs. potentially digestible fiber) in relation to energy,
nitrogen balance and milk performance and enteric and manure methane
productions (Experiment 2).
3. To study the effect of substitution of barley grain in the mixed diet by dry orange pulp or soybean hulls on energy partitioning, methane emissions, nitrogen and carbon balance and milk performance in dairy goats (Experiment 3).
EXPERIMENT I: Effect of replacing dietary corn with beet pulp on energy partitioning, substrate oxidation and methane production in lactating dairy goats

EXPERIMENT I: Replacement of corn grain with beet pulp

Effect of replacing dietary corn with beet pulp on energy partitioning, substrate oxidation and methane production in lactating dairy goats

C. Ibáñez\textsuperscript{A}, M. C. López\textsuperscript{A}, P. Criscioni\textsuperscript{A} and C. Fernández\textsuperscript{A\textsuperscript{B}}

\textsuperscript{A}Institute for Animal Science and Technology, Polytechnic University of Valencia. 46022 Valencia, Spain.

\textsuperscript{B}Corresponding author. E-mail: cjfernandez@dca.upv.es
EXPERIMENT I: Replacement of corn grain with beet pulp

1.1. Abstract

The objective of this experiment was to study the effect of substitution of corn by beet pulp on energy partitioning, substrate oxidation, nitrogen and carbon balance and milk performance in dairy goats during late lactation. Twelve multiparous lactating Murciano-Granadina goats were fed two diets. Six goats were fed a mixed ration with 310 g/kg DM of ground corn (diet CORN) and in the other diet the corn was substituted with 302 g/kg DM of beet pulp (diet BP) in a cross-over design. No significant differences between diets were observed for milk production (1.36 kg/d, on average) and differences were found for milk fat (5.39% and 4.21% for BP and CORN, respectively). The MEI was higher (P < 0.05) in diet CORN than BP (1320 vs. 1044 kJ/kg$^{0.75}$ BW, respectively). The difference in methane emissions between treatments was significant (P < 0.05) with values of 92 vs. 61 kJ/kg$^{0.75}$ BW for BP and CORN, respectively, indicating that greater level of starch in diet reduce the methane production. Replacing corn by BP reduced significantly the energy body fat deposition (300 vs. 44 kJ/kg$^{0.75}$ BW for CORN and BP diets, respectively). This reduction in energy retention for diet BP did not compromise milk yield and milk energy output.

Keywords: goat, heat production, substrate oxidation, beet pulp, methane.
1.2. Introduction

Cereals grains are a substantial feed resource for dairy ruminants in European and American production systems. The Spanish production system (Interal, 2008; Calsamiglia et al., 2009) is based on high use of concentrate (40-70%), using mixed diets instead of whole forage rations. High levels of concentrate (mainly cereal grain) in the diet are common practice in Spain due to the lack of pasture. High concentrate feeding usually increases milk production and depresses milk fat in high producing cows (Grainger and Beauchemin, 2011). Optimal ruminal fermentation from high concentrate diets probably can be achieved by diluting starch with a non forage carbohydrate source that is less rapidly fermented, produces less propionate, without reducing ruminal pH. Beet pulp by its highly digestible fiber content, is expected to produce a favourable rumen fermentation pattern and proper milk fat synthesis (Mahjoubi et al., 2009). The neutral detergent fiber (NDF) in beet pulp can be digested more quickly than forage NDF, and pectins, which are not recovered in NDF, are degraded more rapidly than cellulose and hemicellulose. Although, these differences depend on the type of forage, and forages such as chicory and some brassicas have NDF that is at least as rapidly degraded as the one in beet pulp. Sugar beet pulp can sustain similar microbial crude protein (CP) production compared with corn despite lower non fibrous carbohydrates (NFC) concentration, consequently, beet pulp can support similar milk yield compared with other energy sources higher in NFC (Mansfield et al., 1994). Nevertheless the information about methane (CH$_4$) emission is scarce.

Changing diet composition and/or intake can influence partitioning of nutrients towards the mammary gland rather than body tissues. In an investigation of the effect of lipogenic and glucogenic feeds on nutrient partitioning and energy balance, Van Knegsel et al. (2007) showed that cows fed a lipogenic diet partitioned more energy to milk than cows fed a glucogenic diet, and that energy mobilized from body fat tended to be higher in cows fed a lipogenic diet vs. cows fed the glucogenic diet. To do this is necessary to measure possible changes in energy metabolism and dissipation. Two indirect
methods to determine the energy metabolism of animals are available from previous decades (McLean and Tobin, 1987). Indirect calorimetry (IC method) is based on measurements of oxygen (O₂) consumption, carbon dioxide (CO₂), CH₄ production and nitrogen (N) excretion in urine. The carbon (C) and N balance (CN method) is another indirect procedure that measures the intake and excretion of C and N. The IC method was used to determine the associated heat production (HP), and CN method was used to calculate the retention of energy (RE), assuming that all energy is retained either as fat or protein.

Based on these premises, the aim of this experiment was to study the effect of substituting corn with beet pulp in the diet of lactating goats on milk yield, energy partitioning, substrate oxidation and CH₄ emissions. As few data are available regarding HP estimated with the two methods in dairy goats, both will be shown.

1.3. Materials and methods

1.3.1. Animals and feeding

The experimental procedure was approved by the Animal Use and Care Committee of the Polytechnic University of Valencia (Spain) and followed the codes of practice for animals used in experimental works proposed by the European Union (2003). Twelve multiparous mature Murciano-Granadina goats, which were 4 years of age, of similar body weight (47.0 ± 2.5 kg BW) in late lactation were randomly fed a diet in which one group was given a diet containing 30% ground corn (CORN) and the other group was given a diet in which the corn was substituted with beet pulp (BP) in a cross-over design. The forage to concentrate in the ration was 37:63 and the concentrate was pelleted. Forages were alfalfa hay and barley straw, and they were cut into 2.5-cm pieces (Skiold Saby A/S, Kjeldgaardsvej, DK 9300). Ingredients and chemical composition of diets are shown on Table 1.1. The two whole mixed rations had an average energy value of 17.46 MJ of gross energy (GE)/kg DM and a CP value of 15.2% on average, DM basis. The starch concentration were 29.03% DM and 14.4% DM for CORN and BP, respectively. Both diets were optimized according
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to the recommendations of Lachica and Aguilera (2003) and Calsamiglia et al. (2009) for goats during lactation. Goats were fed ad libitum at 110% of consumption with the experimental diets and they were allocated in pens for 10 days. Half the daily ration was offered at 0800 and half at 1600 hours, respectively. Goats had free access to water.

1.3.2. Experimental schedule and measurements

Goats were allocated to individual metabolism cages at thermoneutrality (20-23°C) during another 10 days of adaptation. Then apparent total tract digestibility, energy and CN balance were determined; feed intake, refusal and total fecal, urine and milk output were recorded daily for each goat during a 5-day period, as well as BW at the beginning and end of the period. Feces were collected in wire-screen baskets placed under the floor of the metabolism crates and urine was collected through a funnel into plastic buckets containing 50 mL of 10% (vol/vol) H₂SO₄ to acidify the urine. Representative samples (20%) of diet, feces and urine were collected on 5 consecutive days, stored at -20°C and pooled for chemical analysis. The goats were milked once daily at 0700 hours with a portable milking machine (Flaco, model DL-170, J. Delgado S.A., Ciudad Real, Spain). Immediately after the apparent digestibility samples were collected, the individual milk yield was weighed and, after mixing, a sample of 10% was put in a bottle with 20 mg of potassium dichromate as preservative and stored at 4°C before analyses. Ruminal fluid samples were collected by stomach tube before the morning feeding on the last day of apparent digestibility trial. A 5-mL subsample of strained ruminal fluid was acidified to pH 2.0 and mixed with 50% H₂SO₄ and frozen until analysis for volatile fatty acids (VFA).

After apparent digestibility and balance experiment were finished, gas exchange was measured for 15 min/h per goat each 3 hour for 24 h (8 measures/d with 4 goats/h). The gas exchange was measured using a face mask, which was fixed to the head of the goat by a rubber band. Full adaptation of the animals to the face mask was vital for the success of the experiment. The respirometry system has two separate sampling lines. The main line sucked air
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through a face mask consisting of a plastic funnel (15 by 2.5 inner diameter, and 25 cm long) with an internal rubber draught excluder glued to the internal border for a better fit to the animal’s face and fixed by a rubber band around the head. The funnel was attached to a 2.5-cm (inner diameter) PVC corrugated tube equipped with an air filter to keep dust out. Total airflow through the system was measured by a mass flow meter (Thermal Mass Flowmeter Sensyflow VT-S, ABB, Alzenau, Germany) and air suction was by a centrifugal fan (CST60 Soler Palau Inc., Parets del Vallès, Barcelona, Spain). The mass flow meter had a digital output for computer connection. A subsampling line (polyethylene tubing, 5-mm inner diameter) was located after the mass flow meter to take a sample of gas from the main line using a membrane pump (ABB). It was attached to a rotameter (DK800, ABB) with valve to set up the desirable flow rate into the gas analyzer (Easyflow 3020 model, ABB). The CH$_4$ and CO$_2$ were measured by using the infrared principle with an upper limit at 0.15 and 1.5%, respectively; O$_2$ was measured by using the paramagnetic principle covering the range 19-21% and was equipped with an atmospheric compensation module to offset changes in pressure. The analyzer was an autocalibrated model and no master cylinders were required. It was monitored with a digital panel meter. This analyzer was controlled by MODBUS with a PC (Fujitsu Siemens Lifebook Series, Pentium 4 laptop, Munich, Germany) under a LabVIEW (http://www.ni.com/labview, accessed 20 November 2013) environment to save the data in the hard disk. Detailed of the mobile open-circuit respirometry system used for these measurements was described by Fernández et al. (2012a).

The whole system was calibrated by injecting pure N$_2$, CO$_2$ and CH$_4$ into the mask (McLean and Tobin, 1987) determined gravimetrically using a precision scale. Calibration factors were calculated according to Brockway et al. (1971). The CH$_4$ and CO$_2$ production and O$_2$ consumption were calculated as described Aguilera and Prieto (1986). An initial atmospheric air sample was collected and the gas concentrations were used as reference for calculations.
1.3.3. Chemical analyses

Feed, feed refusal and feces samples were first dried in a forced air oven at 55°C for 48 h then ground to pass a 1-mm screen. Urine was dried by lyophilisation. Chemical analyses of the diet, refusals and feces were conducted for DM, ash, ether extract (EE) and CP following AOAC (2000). DM of diets and feces was determined by oven-drying at 102 ± 2°C for 24 h and organic matter content (OM) was determined by incineration in an electric muffle furnace at 550°C for 6 h. The EE was extracted with petroleum ether after acid hydrolysis to recover saponified fat (Soxtec System HT Tecator, Hillerød, Denmark; 1047 Hydrolyzing Unit and 1043 Extraction Unit). The acid detergent fiber (ADF) and NDF were measured in an ANKOM Fiber Analyzer (A220, ANKOM Technologies, Fairport, NY, USA) according to Van Soest et al. (1991) using sodium sulphite and α-amylase. NFC content of diets was calculated by difference method based on chemical analysis of individual feeds as NRC (2001) shown; NFC = 100 – NDF – ash – CP – EE. The GE content of the dried samples (feed, feces, urine and milk) was analyzed in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK). Starch content was determined by enzymatic method (α-amylase obtained from Sigma-Aldrich, Steinheim, Germany) according to Batey (1982). The CN was analyzed by the Dumas principle (TruSpec CN; LECO Corporation, St. Joseph, MI, USA). Multiplying N by a factor of 6.25 and 6.38 converted the results to CP for feed and milk, respectively. Milk composition (fat, protein, lactose, and total milk solids content) was analyzed with an infrared analyzer (MilkoScan FT120 Foss Electric, Hillerød, Denmark).

Determination of VFA was based on the method previously described by Jouany (1982). Samples were filtered through 0.45-µm cellulose syringe filters. One-hundred microliters of an internal standard solution (0.4 g of 4-metil-valeric acid diluted in 100 mL of deionized water) and 0.1 mL of a preservative (a mix of 5% H₃PO₄ and 1% ClHg in deionized water) were added to 0.8 mL of filtrate. One µL from each sample was injected in a gas chromatograph (Fisons 8000 series, Milan, Italy) equipped with a split/splitless injector and FID detector. The VFA separation was performed in a DB-FFAP capillary column (30 m by 0.25 mm by
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0.25 µm of film thickness) J&W Scientific (USA). The carrier gas was N\(_2\) at a constant pressure of 120 kPa. Both detector and injector temperatures were set at 245 °C. The initial oven temperature was set at 115 °C held for 5 min and increased to 230 °C at 8.5 °C/min and finally maintained at that temperature for 10 min. VFA were identified by comparing their retention times with a standard (46975-U de Supelco, Bellefonte, PA, USA).

1.3.4. Calculations

The metabolizable energy intake (MEI) was calculated as the difference between GE intake and energy losses in feces, urine and CH\(_4\) (with an energy equivalent value of 39.5 kJ/L CH\(_4\); Brouwer, 1965).

The efficiency of use of ME for lactation when there is no change in body energy stores was calculated according to ARC (1980). Energy lost from the body, indicating mobilisation of body fat reserves in support of milk secretion, was assumed to be used for milk synthesis with an efficiency of 0.84 and the concomitant energy storage during lactation was taken to be 0.95 times the milk secretion efficiency. Consequently, the corrected milk energy was estimated as E\(_{\text{milk}}\) (ARC 1980). The efficiency of use of ME for milk production (k) was calculated as E\(_{\text{milk}}\) / (ME - MEm), and MEm was obtained from the estimation of Aguilera et al. (1990) for Granadina goats from both positive and negative energy retentions (401 kJ/kg\(^{0.75}\) BW).

The HP was calculated by the IC method according to Brouwer (1965) for O\(_2\) consumption (L/day), CO\(_2\) and CH\(_4\) production (L/day) and urine-N (Nur, g/day) as:

\[
\text{HP (kJ) = (16.18} \times \text{O}_2 + (5.02} \times \text{CO}_2) - (2.17} \times \text{CH}_4) - (5.99} \times \text{Nur}
\]

The RE was calculated as the difference between MEI and HP. The body retention of energy (RE\(_{\text{body}}\)) was the difference between RE and milk energy (E\(_{\text{milk}}\)).

The energy associated with oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF) was calculated by the method of Brouwer (1958) and
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Chwalibog et al. (1997) for ruminants. The production of VFA from carbohydrate fermentation is followed by CO$_2$ and CH$_4$ production. Fahey and Berger (1988) demonstrated CO$_2$/CH$_4$ ratio of 3/1 for high grain diets and 1.7/1 and high forage diets, respectively. The CO$_2$ production from oxidation (CO$_2$x) was calculated as CO$_2$ - (3 × CH$_4$) for CORN, and CO$_2$ - (1.7 × CH$_4$) for BP. The calculations were carried out as follows:

\[ OXP = 6.25 \times Nur \times 18.42 \text{ (kJ/g)} \]

\[ OXCHO = \left[ (-2.968 \times O_2) + (4.174 \times CO_{2x}) - (2.446 \times Nur) \right] \times 17.58 \text{ (kJ/g)} \]

\[ OXF = \left[ (1.719 \times O_2) - (1.719 \times CO_{2x}) - (1.963 \times Nur) \right] \times 39.76 \text{ (kJ/g)} \]

Then, the HP from oxidation was:

\[ HPx \text{ (kJ)} = (16.18 \times O_2) + (5.02 \times CO_{2x}) - (5.99 \times Nur) \]

Gases were expressed in L/day and Nur in g/day. The non-protein respiratory quotient (RQnpx) from oxidation of nutrients was determined as:

\[ RQnpx = \left[ CO_{2x} - (Nur \times 6.25 \times 0.774) \right] / \left[ O_2 - (Nur \times 6.25 \times 0.957) \right] \]

In the CN method, the C balance gave the total amount of C retained in the body and the amount of C retained in fat was calculated by subtracting the amount of C retained in protein determined by the N balance. Assuming an energy equivalent of 39.76 kJ/g of fat and a content of 0.767 gC/g of fat, and 23.86 kJ/g of protein and 0.16 gN/g and 0.52 gC/g of protein, the energy retained (kJ) in protein (RE$_{protein}$) and fat (RE$_{fat}$) was calculated, respectively, as

\[ RE_{protein} = N \text{ balance (g)} \times 6.25 \times 23.86, \text{ and RE}_{fat} = [C \text{ balance (g)} - N \text{ balance (g) } \times 6.25 \times 0.52] \times 1.304 \times 39.76. \]

The total retention of energy (RE$_{total}$, kJ) was calculated as RE$_{total}$ = RE$_{protein}$ + RE$_{fat}$. The retention energy in milk (RE$_{milk}$, kJ) from C and N in milk was determined using the following expression of McLean and Tobin (1987): 51.80 x gC$_{milk}$ - 19.38 x gN$_{milk}$. Therefore, the heat production determined by CN method (HP$_{CN}$) is: HP$_{CN}$ = MEI - TE$_{total}$ - RE$_{milk}$. 

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1.3.5. Statistical analyses

The effects of replacing dietary corn with beet pulp on intake, digestibility, milk yield, balances (energy, C and N) and CH₄ production were analyzed using the mixed model (proc MIXED) from SAS software (2001). The experiment was conducted as a cross-over design; each goat received both treatments in two periods. Goat served as the experimental unit for all data. The model for the dependent variables included the fixed effect of diet, period and their interaction, with goat as random effect. The following statistical model was used: 

\[ Y = \mu + D + T + D \times T + \text{goat} + \varepsilon, \]

where Y is the dependent variable, \( \mu \) is the overall mean, D and T are the fixed effects of diet and period of time, DxT the interaction, goat is the random effect of goat and \( \varepsilon \) is the random error. Least square means are reported throughout and differences were considered significant at \( P < 0.05 \).

1.4. Results and discussion

There was no significant effect for the fixed effect period and its interaction with diet throughout the experiment, so the Tables report only the effect of diet. The average value obtained for the calibration factor by releasing a known volume of N\(_2\) into the respirometry system was 1.0065 ± 0.01307 (L/L). The calibration factor for CO\(_2\) and CH\(_4\) were 1.052 ± 0.0033 (L/L) and 0.945 ± 0.0042 (L/L), respectively. The values close to one confirmed the absence of leaks and good performance of the system.

1.4.1. Animal performance, digestibility and rumen parameters

Table 1.2 shows the main production results of the goats in the experiment. The difference in dry matter intake (DMI) between treatments was not significant (\( P < 0.09 \)), with a higher numerical value for diet CORN than BP (1.89 vs. 1.63 kg/day, respectively). The higher feed intake registered on diet CORN was presumably a result of a lower retention time of this diet in the gastrointestinal tract, due to its small bulk effect because of the lower quantity of barley straw on diet CORN than BP (Grainger and Beauchemin, 2011).
Average milk yield was 1.32 L/goat day and diet had no effect on milk yield. Therefore, BP could be support milk yield, in the short term, similar to other feed energy sources high in NFC (Voelker and Allen, 2003), obviously under our experimental conditions: late lactation and same type of mixed diet. The NFC was 41 and 34% for CORN and BP diets, respectively, both expressed on DM basis. Bhattacharya and Lubbadah (1971) replaced 73% of corn with BP and found no differences in milk yield. An increase in NDF : starch ratio (1.0-2.5 for CORN and BP, respectively) did not increase significantly the milk energy output in our study (Table 1.5), although numerically higher milk yield was found for CORN than BP, more milk energy output was found for BP than CORN. Increased milk fat content is common when dietary NDF concentrations increase at the expense of starch. Significant differences ($P < 0.05$) were observed for milk fat (5.39 and 4.21% for BP and CORN, respectively), and greater values of acetic acid on rumen liquor were found in diet BP than CORN (see below, Table 1.4). Van Knegsel et al. (2007) showed that milk fat level was usually elevated after feeding extra lipogenic nutrients. These authors replaced 27% of corn with 29% of BP in dairy cows. Mahjoubi et al. (2009) adding BP to dairy cows at late lactation in substitution of barley grain, increased milk energy output mainly because of increased milk fat output. Milk protein content was not statistically different between treatments (4.18% on average). It seems that goats consuming CORN were probably overfed with starch, considering their stage of lactation and milk yield; this could be one of the reasons for the higher fat deposition (Table 1.7) for CORN than BP. However, BP diet still had enough energy concentration to maintain milk yield.

Total apparent tract digestibility values were numerically greater for diet CORN than BP (Table 1.3). The higher level of starch and lower level of fiber in diet CORN appeared to be the main factor responsible for the decrease of DM, OM, EE, NDF and energy apparent digestibility in diet BP. Significant effect was observed ($P < 0.05$) for OM, NDF and EE digestibility. The lower values for BP probably due to the greater NDF and ADF content of diet BP than CORN and the simple fact that diet CORN has 51% more starch than diet BP. Different authors reviewed the high fermentation and digestibility of starch from cereal
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grain: so, Waldo (1973) and Huntington (1997) reported ruminal digestibility of the whole corn to be 60% and total tract digestibility as 92%.

Rumen fermentation parameters obtained are shown in Table 1.4. No effect of period of time is shown because rumen liquid extraction was done only in the first part of the trial. Goats fed the CORN diet had greater ammonia N concentration (P < 0.05) than BP (24.59 vs. 12.69 mg/dL, respectively), probably linked to the lower N expenditure by ruminal bacteria to synthesise microbial protein (Casper et al., 1999). These authors also report that these differences seem to be an asynchrony between rumen carbohydrate degradability and rumen degraded protein to maximise microbial protein synthesis. Bava et al. (2001) with Saanen diary goats at mid lactation found ammonia N values of 28.8 and 53.3 mg/dL for diets with 14.8 and 21.4% CP (on DM basis), respectively. A significant increase of acetic acid in the rumen when goats fed the more fibrous diet was observed (68.7 and 62.9 mol/100 mol for BP and CORN diets, respectively). The rest of VFA showed no significant differences between diets. Therefore, the BP diet had low starch content (14 vs. 29%) and the energy it provides would be mainly in the form of highly digestible fiber (diet BP has an estimation of sugars + pectins of -19% and CORN diet of -11%).

1.4.2. Energy metabolism

The IC method can be expected to yield systematically higher values for HP than the CN method (Tables 1.5 and 1.7). However, both methods gave very close values accounting for 684 and 715 kJ/kg^{0.75} BW/day on average for CN and IC methods, respectively, and no significant differences were found when both methods were compared (P = 0.48). Both methods are partially dependent on each other; the agreement between them may be an indication of the absence of errors in methodology (Blaxter, 1967; Christensen et al., 1988). Discrepancies \(((HP - HP_{CN})/MEI) \times 100\) averaged 2.6% when expressed as a percentage of the MEI, a rather satisfactory value taking into account the considerable amount of technical and analytical work involved. Aguilera and Prieto (1986), with wethers confined in respirometry chambers for 24 h and fed at around maintenance
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level, obtained an average discrepancy of 1.8%, while Fernández et al. (2012a), with goats following a similar sampling schedule to that in the present study, reported a value of 0.9%.

Daily energy balance estimated by the IC method is displayed in Table 1.5. The MEI was higher ($P < 0.05$) for diet CORN than BP (1320 vs. 1044 kJ/kg^{0.75} BW, respectively), mainly because the GE intake was greater and less energy was lost in CH₄. The CH₄ production for the BP diet was significant and higher ($P < 0.05$) than for the CORN diet (92 vs. 61 kJ/kg^{0.75} BW, respectively), indicating that increasing the level of starch in diet reduces the CH₄ production, as reported by different authors and reviewed by Grainger and Beauchemin (2011), and on the other hand, the higher ammonia N observed in the CORN diet as responsible of less fermentative activity and lower CH₄ production. However, Van Knegsel et al. (2007) found no differences in CH₄ production from dairy cows fed a diet contained 27% of corn with regards to a diet with 29% of BP. The measured CH₄ emissions were in the range by other authors using a respiration chamber; Prieto et al. (1990) reported emission ranging from 42 and 63 kJ/kg BW^{0.75} for castrated male Granadina goats, and Aguilera et al. (1990) reported emission regimes from 89 to 117 kJ/kg BW^{0.75} for lactating females, with both diets based on pelleted alfalfa hay and barley, although those studies did not show the starch and fiber levels of the diets. The HP was not significant between diets, with an average value of 715 kJ/kg^{0.75} BW. So, considering HP as a percentage of the MEI, goats fed high starch diets had lower heat expenditure (56 vs. 66% for CORN and BP, respectively).

No significant differences were observed for $E_{\text{milk}}$ content, although numerical greater values were observed for BP than CORN (292 vs. 277 kJ/kg^{0.75} BW, respectively). Kirkland and Gordon (2001) found that the level of milk energy output influences partitioning of ME towards milk production, rather than body tissues, during late lactation, suggesting that energy is first directed towards maintenance of milk production and then to body gain. Therefore, the tissue energy recovered in the body was lower ($P < 0.05$) for the BP than the CORN diet (61 vs. 305 kJ/kg^{0.75} BW). It seems that greater quantities of starch in diet cause
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tissue fat deposition (Tedeschi et al., 2006). Mahjoubi et al. (2009) substituted barley grain with BP in dairy cows at late lactation, and found a reduction of body condition score and body fat thickness.

The efficiency of use of ME for milk production (k) was calculated as milk energy output adjusted to zero energy balance, divided by ME-MEm. MEm was obtained from the estimation of Aguilera et al. (1990) for Granadina goats from both positive and negative energy retentions (401 kJ/kg0.75 BW). No significant differences were observed between diets for k (0.60 on average) and a similar value to that of Aguilera et al. (1990) was found with lactating Granadina goats (k=0.67). Bava et al. (2001) found values ranging from 0.60 to 0.73 for Saanen goats during lactation. Moreover, Tovar-Luna et al. (2010) with Alpine goats during lactation, found values ranging from 0.66 to 0.78. These authors found variability when combining different stages of lactation with level of feed intake, one of the reasons for differences in efficiencies being related to the complexity of biochemical transformation in tissues that are being synthesized or mobilized. Significant differences (P < 0.03) were found for the net energy value of the ration: 8.2 vs. 6.5 MJ/kg DM in CORN and BP diets, respectively. Therefore, although the mixed diets were isoenergetics in terms of GE, the efficiency of energy utilisation were different when it was expressed on net energy bases.

The magnitude of enteric CH4 formation in ruminants depends on type and dietary proportions of different carbohydrates, that is, cellulose, starch, oligo-, di-, monosaccharide (Hindrichsen et al., 2005). It is often claimed that forage-based diets generally result in considerable greater methane production than mixed- or concentrated- based diets (Grainger and Beauchemin, 2011). The amount of CH4 emission was significant (P < 0.01) and greater for BP than CORN (29.7 vs. 19.6 g/day). The CH4 production in relation to ingested organic matter (OMI) reflected the differences in proportion between concentrates and roughages in the diets. The ratio CH4 and OM intake was used as an indicator of fermentation level due to increase VFA production, more CO2 is originated from fermentation processes and subsequently less from nutrient oxidation at the tissue level. The CH4 production increased (P < 0.01) from 12 to 22 g/kg OMI indicating increased
in fermentation and greater CH$_4$ production related to greater fiber on the diet BP, and lack of fermentation in diet CORN (higher values of ammonia N).

1.4.3. Oxidation of nutrients

CO$_2$ production is derived from nutrient oxidation and rumen fermentation. The separation between these two components is necessary to calculate the substrate oxidation in ruminants and determine the proportion of substrate oxidation supporting the total HP associated with oxidative processes; HPx. The production of VFA from carbohydrate fermentation is followed by CO$_2$ and CH$_4$ production. The proportional contribution to HPx due to oxidation of nutrients with the two diets was shown in Table 1.6.

In ruminants, lipogenic nutrients originate either from fiber or dietary fat or from body reserves. Glucogenic nutrients originate from starch escaped from rumen degradation or gluconeogenesis (Van Knevel et al., 2007). The OXCHO (384 kJ/kg$^{0.75}$ BW) is higher than OXF (274 kJ/kg$^{0.75}$ BW) for the diet with a greater content of starch, and OXF (329 kJ/kg$^{0.75}$ BW) is greater than OXCHO (257 kJ/kg$^{0.75}$ BW) for diet rich in fibrous ingredients. The OXCHO was statistically different between CORN and BP: 384 vs. 257 kJ/kg$^{0.75}$ BW, respectively. It seems that the greater OXCHO and lower OXF found in diet CORN (and reciprocal for BP) was in part responsible for the higher recovered tissue energy in the body; 305 vs. 61 kJ/kg$^{0.75}$ BW for CORN and BP, respectively (Table 1.5). These results suggest that glucogenic nutrients stimulate body fat deposition and the partitioning of ME into body tissues. Therefore, the HPx derived from OXF increased as the value of MEI decreased. As we observed in Table 1.5, the increasing value of CH$_4$ regards to OMI is indicative of greater ruminal fermentation in diet BP. With increasing fermentation less glucose was absorbed directly and oxidized as OXCHO, while more carbohydrate was converted to VFA and oxidized as OXF. Therefore, most of the HPx derived from OXF (49%) was for diet BP and from OXCHO (48%) for diet CORN. Few studies relating to oxidation of nutrients are available for ruminants and especially for small ruminants. Chwalibog et al. (1997) performed a study with calves with positive RE as fat pointed out that part of OXF should originate
from ingested carbohydrate, mainly fiber. On the other hand, Fernández et al. (2012b) found in Manchega sheep that in fasting conditions most of the HPx was due to OXF (93%), and OXCHO was increasing with a feeding level up to a stable value of 51%.

All RQnpx values (Table 1.6) were below 1.0, indicating fat oxidation (Chwalibog et al., 1997). Moreover, there was clear tendency of decreasing RQnpx with increasing CH₄ production. As we mentioned in Table 1.5, the CH₄ production increased from 19.6 to 29.7 g/day for diet CORN and BP, respectively. This increased in CH₄ production was followed by an increased in the OXF of 43-49% of HPx for CORN and BP diets, respectively, with reciprocal values for OXCHO (from 48% to 37%). The reduction in RQnpx was consistent with the switch between OXCHO and OXF as less carbohydrate and more fat was oxidized. The values of OXCHO and OXF were directly dependent on the fermentation level. With increasing fermentation less glucose was absorbed and oxidized as OXCHO, while more carbohydrate was converted to VFA and oxidized as fat, being in accordance with the values of RQnpx decreasing from 0.97 to 0.81.

1.4.4. Nitrogen and carbon balance

The daily N and C balance and the calculated tissue recovered energy, as protein and fat, is displayed in Table 1.7. Due to no significant effect in DMI between diets, no significant differences were found in N intake, milk N and N retention. Therefore, energy retention as protein was positive for both treatments, as Van Knegsel et al. (2007) found in dairy cows replacing mainly corn with sugar beet pulp. No significant differences were found either, for C intake, C in feces and urine excretion, CO₂ and milk C. Diet CORN had lower CH₄ than BP (P < 0.05) with values of 1.10 and 1.85 g/kg⁰.⁷⁵ BW, respectively. Values of N and C balances were converted to energy units and no significant differences were observed between diets for REprotein, but diets tended (P = 0.08) to be different (57 vs. 32 kJ/kg⁰.⁷⁵ BW, in CORN and BP diets, respectively). Significant differences (P < 0.02) were found for REfat (300 vs. 44 kJ/kg⁰.⁷⁵ BW, for CORN and BP, respectively), and the total RE was positive for the two treatments with significant differences (P <
0.05) and values of 357 and 76 kJ/kg\(^{0.75}\) BW, for CORN and BP, respectively. A diet higher in starch seems to increase the amount of fat synthesis in the body (Corbett and Freer, 2003) and therefore energy retention. Therefore, energy retention tended to be lower for goats fed lipogenic diets compared with that for goats fed the glucogenic diet. Thus, goats fed the lipogenic diet probably mobilized more energy; so, in our study greater OXF and less tissue energy deposited as fat were observed in diet BP.

In the present study, goats fed the CORN diet seemed to lower the priority of milk fat production as indicated by lower milk energy output, and increased the priority of energy retention in body reserves as indicated by the increased energy balance. In the present experiment, dietary energy source did not alter the body protein balance, but specifically the body fat balance was greater with feeding a more starchy diet.

1.5. Conclusions

This study confirmed the hypothesis that energy partitioning between milk and body tissue, can be altered by feeding diets that differ in concentration of lipogenic (BP) and glucogenic (CORN) nutrients. Methane and daily milk fat yield were higher in goats fed the BP diet compared with a corn-based diet and consequently the higher milk energy resulted in a tendency for less energy to be partitioned into body fat. Results suggest that inclusion of BP in the diet of late lactation goats can reduce, in short term, the energy retention without compromising milk yield and milk energy output.

1.6. Acknowledgements

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EXPERIMENT I: Replacement of corn grain with beet pulp

1.7. References


Brouwer, E. (1958) On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat metabolized in ruminants, from data on gaseous exchange and urine-N. In: Proceedings of the 1st EAAP
EXPERIMENT I: Replacement of corn grain with beet pulp


EXPERIMENT I: Replacement of corn grain with beet pulp


Mahjoubi, E., Amanlou, H., Zahmatkesh, D., Gheleich, M., Aghaziarati, N. (2009) Use of beet pulp as a replacement for barley grain to manage body
EXPERIMENT I: Replacement of corn grain with beet pulp

condition score in over-conditioned late lactation cows. Animal Feed Science and Technology 153, 60-67. doi:10.1016/j.anifeedsci.2009.06.009


EXPERIMENT I: Replacement of corn grain with beet pulp


EXPERIMENT I: Replacement of corn grain with beet pulp

Table 1.1. Ingredients (g/kg DM) and chemical composition of diets (% of DM): corn diet (CORN) replaced with beet pulp (BP).

<table>
<thead>
<tr>
<th>Ingredients (g/kg DM)</th>
<th>CORN</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa hay</td>
<td>353</td>
<td>216</td>
</tr>
<tr>
<td>Barley straw</td>
<td>21</td>
<td>127</td>
</tr>
<tr>
<td>Corn</td>
<td>310</td>
<td>-</td>
</tr>
<tr>
<td>Barley</td>
<td>218</td>
<td>217</td>
</tr>
<tr>
<td>Beet pulp</td>
<td>-</td>
<td>302</td>
</tr>
<tr>
<td>Soy meal 44% crude protein</td>
<td>65</td>
<td>110</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Bicalcium phosphate</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin mineral premixC</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical composition (% of DM)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>88.1</td>
<td>88.5</td>
</tr>
<tr>
<td>Organic matter (OM = 100 - ash)</td>
<td>88.8</td>
<td>88.0</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>14.7</td>
<td>15.7</td>
</tr>
<tr>
<td>Ether extract</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>30.7</td>
<td>35.7</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>11.3</td>
<td>18.5</td>
</tr>
<tr>
<td>Starch</td>
<td>29.0</td>
<td>14.4</td>
</tr>
<tr>
<td>Carbon</td>
<td>41.3</td>
<td>41.7</td>
</tr>
<tr>
<td>NFC</td>
<td>40.7</td>
<td>33.7</td>
</tr>
<tr>
<td>Sugar + pectins</td>
<td>11.4</td>
<td>19.3</td>
</tr>
<tr>
<td>Gross energy (MJ/kg DM)</td>
<td>17.4</td>
<td>17.5</td>
</tr>
</tbody>
</table>

aCORN = corn diet; BP = beet pulp diet; DM = dry matter; OM = organic matter.
obAll ingredients were pelleted with exception of alfalfa hay and barley straw. Chemical composition shown whole mixed ration.
cProvided by NACOOP S.A. España. (ppm or UI per kilogram of premix): Se, 40; I, 250; Co, 80; Cu, 3000; Fe, 6000; Zn, 23400; Mn, 29000; S, 60000; Mg, 60000; vitamin A, 2000000 UI; vitamin D3, 400000; vitamin E, 2000 ppm; nicotinic acid, 10000; choline, 20300.
dNFC = 100 - [neutral detergent fiber + crude protein + ash + ether extract]; non fibrous carbohydrate.
eSugars + pectins = NFC - starch.
**Table 1.2.** Body weight (kg), intake (kg/day) and daily milk production (mL/day) and composition (%) of milk produced by Murciano-Granadina goats (n = 24) during late lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items</th>
<th>CORN</th>
<th>BP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>46.8</td>
<td>47.1</td>
<td>1.74</td>
<td>0.93</td>
</tr>
<tr>
<td>Dry matter intake (kg/day)</td>
<td>1.89</td>
<td>1.63</td>
<td>0.073</td>
<td>0.09</td>
</tr>
<tr>
<td>Milk yield (L/day)</td>
<td>1.35</td>
<td>1.29</td>
<td>0.903</td>
<td>0.75</td>
</tr>
<tr>
<td>Milk composition (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>14.6</td>
<td>14.9</td>
<td>0.25</td>
<td>0.58</td>
</tr>
<tr>
<td>Fat</td>
<td>4.2</td>
<td>5.4</td>
<td>0.16</td>
<td>0.05</td>
</tr>
<tr>
<td>Protein</td>
<td>4.2</td>
<td>4.1</td>
<td>0.11</td>
<td>0.55</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.7</td>
<td>4.7</td>
<td>0.08</td>
<td>0.92</td>
</tr>
</tbody>
</table>

CORN = corn diet; BP = beet pulp diet; SEM = standard error of the mean.
**EXPERIMENT I**: Replacement of corn grain with beet pulp

**Table 1.3.** Influence of diet on the apparent digestibility coefficients (%) of different dietary components when were fed corn (CORN) and beet pulp (BP)-based diets during late lactation in Murciano-Granadina goats (n = 24).

<table>
<thead>
<tr>
<th>Items (%)</th>
<th>CORN</th>
<th>BP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM^</td>
<td>79.8</td>
<td>74.2</td>
<td>1.24</td>
<td>0.06</td>
</tr>
<tr>
<td>Organic matter</td>
<td>80.2</td>
<td>75.5</td>
<td>1.22</td>
<td>0.05</td>
</tr>
<tr>
<td>Crude protein</td>
<td>76.6</td>
<td>75.7</td>
<td>1.53</td>
<td>0.78</td>
</tr>
<tr>
<td>Ether extract</td>
<td>80.3</td>
<td>59.6</td>
<td>3.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>58.9</td>
<td>46.4</td>
<td>1.86</td>
<td>0.05</td>
</tr>
<tr>
<td>Starch</td>
<td>98.1</td>
<td>99.6</td>
<td>0.92</td>
<td>0.89</td>
</tr>
<tr>
<td>Energy</td>
<td>78.5</td>
<td>73.3</td>
<td>1.33</td>
<td>0.07</td>
</tr>
</tbody>
</table>

CORN = corn diet; BP = beet pulp diet; SEM = standard error of the mean.

^DM = dry matter.
**EXPERIMENT I**: Replacement of corn grain with beet pulp

Table 1.4. Rumen parameters: ammonia nitrogen (NH₃ N, mg/dL) and volatile fatty acids (VFA, mol/100 mol) of Murciano-Granadina goats (n = 24) during late lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items</th>
<th>CORN</th>
<th>BP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃ N^a (mg/dL)</td>
<td>24.59</td>
<td>12.69</td>
<td>4.46</td>
<td>0.04</td>
</tr>
<tr>
<td>Volatile Fatty Acids (mol/100 mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>62.9</td>
<td>68.7</td>
<td>0.79</td>
<td>0.01</td>
</tr>
<tr>
<td>Propionic</td>
<td>15.3</td>
<td>13.1</td>
<td>1.03</td>
<td>0.15</td>
</tr>
<tr>
<td>Isobutyric</td>
<td>2.4</td>
<td>3.7</td>
<td>0.17</td>
<td>0.44</td>
</tr>
<tr>
<td>Butyric</td>
<td>15.1</td>
<td>9.8</td>
<td>0.25</td>
<td>0.08</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>2.9</td>
<td>3.5</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>N-valeric</td>
<td>1.3</td>
<td>1.0</td>
<td>0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>N-caproic</td>
<td>0.1</td>
<td>0.1</td>
<td>0.03</td>
<td>0.58</td>
</tr>
</tbody>
</table>

CORN = corn diet; BP = beet pulp diet; SEM = standard error of the mean.
^aNH₃ N = ammonia nitrogen
Table 1.5. Daily energy balance (kJ/kg\(^{0.75}\) BW) of Murciano-Granadina goats (n = 24) during late lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items(^a)</th>
<th>CORN</th>
<th>BP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEI</td>
<td>1871</td>
<td>1595</td>
<td>84.46</td>
<td>0.05</td>
</tr>
<tr>
<td>E(_{\text{feces}})</td>
<td>434</td>
<td>411</td>
<td>39.19</td>
<td>0.71</td>
</tr>
<tr>
<td>E(_{\text{urine}})</td>
<td>56</td>
<td>48</td>
<td>2.53</td>
<td>0.11</td>
</tr>
<tr>
<td>E(_{\text{methane}})</td>
<td>61</td>
<td>92</td>
<td>7.84</td>
<td>0.01</td>
</tr>
<tr>
<td>MEI</td>
<td>1320</td>
<td>1044</td>
<td>59.35</td>
<td>0.02</td>
</tr>
<tr>
<td>HP</td>
<td>738</td>
<td>691</td>
<td>37.05</td>
<td>0.44</td>
</tr>
<tr>
<td>RE</td>
<td>582</td>
<td>353</td>
<td>55.16</td>
<td>0.04</td>
</tr>
<tr>
<td>E(_{\text{milk}})</td>
<td>277</td>
<td>292</td>
<td>19.96</td>
<td>0.12</td>
</tr>
<tr>
<td>RE(_{\text{body}})</td>
<td>305</td>
<td>61</td>
<td>15.26</td>
<td>0.05</td>
</tr>
<tr>
<td>k(_l)</td>
<td>0.65</td>
<td>0.55</td>
<td>0.05</td>
<td>0.76</td>
</tr>
<tr>
<td>NE(_L) (MJ/kg DM)</td>
<td>8.2</td>
<td>6.5</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>CH(_4) (g/day)</td>
<td>19.6</td>
<td>29.7</td>
<td>1.97</td>
<td>0.01</td>
</tr>
<tr>
<td>CH(_4)/OMI (g/kg)</td>
<td>12</td>
<td>22</td>
<td>2.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CORN = corn diet; BP = beet pulp diet; SEM = standard error of the mean.
\(^a\)GEI = gross energy intake; E = energy; MEI = metabolizable energy intake; HP = heat production; RE, retention of energy = MEI - HP; RE\(_{\text{body}}\) = MEI - HP - E\(_{\text{milk}}\); k\(_l\), efficiency of ME for milk production = corrected milk energy/(ME-MEm); NE\(_L\), net energy for lactation = (MEI x k\(_l\)) / DMI; DM = dry matter; OMI = organic matter intake.
Table 1.6. Daily oxidation (kJ/kg$^{0.75}$ BW) of protein (OXP), carbohydrate (OXCHO) and fat (OXF) and their contribution (%) to the heat production from substrates oxidation (HPx) of Murciano-Granadina goats (n = 24) during late lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items$^a$</th>
<th>CORN</th>
<th>BP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPx</td>
<td>718</td>
<td>675</td>
<td>37.20</td>
<td>0.34</td>
</tr>
<tr>
<td>OXP</td>
<td>61</td>
<td>89</td>
<td>8.52</td>
<td>0.10</td>
</tr>
<tr>
<td>OXCHO</td>
<td>384</td>
<td>257</td>
<td>54.38</td>
<td>0.05</td>
</tr>
<tr>
<td>OXF</td>
<td>274</td>
<td>329</td>
<td>32.18</td>
<td>0.10</td>
</tr>
<tr>
<td>OXP/HPx</td>
<td>9</td>
<td>14</td>
<td>1.65</td>
<td>0.06</td>
</tr>
<tr>
<td>OXCHO/HPx</td>
<td>48</td>
<td>37</td>
<td>6.35</td>
<td>0.04</td>
</tr>
<tr>
<td>OXF/HPx</td>
<td>43</td>
<td>49</td>
<td>5.82</td>
<td>0.06</td>
</tr>
<tr>
<td>RQnpx</td>
<td>0.97</td>
<td>0.81</td>
<td>0.01</td>
<td>0.12</td>
</tr>
</tbody>
</table>

CORN = corn diet; BP = beet pulp diet; SEM = standard error of the mean.

$^a$HPx = heat production from oxidation of nutrients (Brouwer, 1958); OXP = oxidation of protein; OXCHO = oxidation of carbohydrates; OXF = oxidation of fat; RQnpx = non protein respiration quotient from oxidation of nutrients.
Table 1.7. Daily C-N balance (g/kg\textsuperscript{0.75} BW), retained energy (RE; kJ/kg\textsuperscript{0.75} BW) and heat production (HP; kJ/kg\textsuperscript{0.75} BW) of Murciano-Granadina goats (n = 24) during late lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items(^a)</th>
<th>CORN</th>
<th>BP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(_{\text{intake}})</td>
<td>2.53</td>
<td>2.43</td>
<td>0.11</td>
<td>0.37</td>
</tr>
<tr>
<td>N(_{\text{feces}})</td>
<td>0.59</td>
<td>0.58</td>
<td>0.06</td>
<td>0.88</td>
</tr>
<tr>
<td>N(_{\text{urine}})</td>
<td>1.05</td>
<td>1.14</td>
<td>0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>N(_{\text{milk}})</td>
<td>0.51</td>
<td>0.51</td>
<td>0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>NB</td>
<td>0.38</td>
<td>0.21</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>RE(_{\text{protein}}) (kJ/kg\textsuperscript{0.75} BW)</td>
<td>57</td>
<td>32</td>
<td>4.39</td>
<td>0.08</td>
</tr>
<tr>
<td>C(_{\text{intake}})</td>
<td>44.44</td>
<td>37.98</td>
<td>1.88</td>
<td>0.12</td>
</tr>
<tr>
<td>C(_{\text{feces}})</td>
<td>10.68</td>
<td>10.01</td>
<td>0.97</td>
<td>0.74</td>
</tr>
<tr>
<td>C(_{\text{urine}})</td>
<td>1.37</td>
<td>1.57</td>
<td>0.10</td>
<td>0.35</td>
</tr>
<tr>
<td>C(_{\text{milk}})</td>
<td>5.44</td>
<td>5.81</td>
<td>0.44</td>
<td>0.71</td>
</tr>
<tr>
<td>CO(_{2})</td>
<td>18.82</td>
<td>17.20</td>
<td>0.94</td>
<td>0.75</td>
</tr>
<tr>
<td>CH(_{4})</td>
<td>1.10</td>
<td>1.85</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>C(_B)</td>
<td>7.03</td>
<td>1.54</td>
<td>1.17</td>
<td>0.03</td>
</tr>
<tr>
<td>RE(_{\text{total}}) (kJ/kg\textsuperscript{0.75} BW)</td>
<td>300</td>
<td>44</td>
<td>12.33</td>
<td>0.02</td>
</tr>
<tr>
<td>RE(_{\text{total}}) (kJ/kg\textsuperscript{0.75} BW)</td>
<td>357</td>
<td>76</td>
<td>19.64</td>
<td>0.02</td>
</tr>
<tr>
<td>RE(_{\text{milk}}) (kJ/kg\textsuperscript{0.75} BW)</td>
<td>272</td>
<td>291</td>
<td>22.07</td>
<td>0.32</td>
</tr>
<tr>
<td>HP(_{\text{CN}}) (kJ/kg\textsuperscript{0.75} BW)</td>
<td>691</td>
<td>677</td>
<td>47.52</td>
<td>0.54</td>
</tr>
</tbody>
</table>

\(^a\)N = nitrogen; NB = nitrogen balance; RE = retention of energy; BW = body weight; C = carbon; CB = carbon balance; RE\(_{\text{total}}\) = RE\(_{\text{protein}}\) + RE\(_{\text{fat}}\); RE\(_{\text{milk}}\) = 51.8g\(_{\text{milk}}\) - 19.38gN\(_{\text{milk}}\) (according to McLean and Tobin 1987); HP = heat production; HP\(_{\text{CN}}\) = MEI - RE\(_{\text{total}}\) - RE\(_{\text{milk}}\).
EXPERIMENT II: Replacement of cereal with low starch fibrous by-products on nutrients utilization and methane emissions in dairy goats

Experiments I: Replacement of cereal with low starch fibrous by-products

Replacement of cereal with low starch fibrous by-products on nutrients utilization and methane emissions in dairy goats

C. Ibáñez1, V.J. Moya2, H. Arriaga3, D.M. López3, P. Merino3 and C. Fernández1*

1Research Group ACUMA, Animal Science Department, Polytechnic University of Valencia, Valencia, Spain
2Institute for Animal Science and Technology, Universitat Politècnica de València, Valencia, Spain
3Neiker-Tecnalia, Basque Institute for Agricultural Research and Development, Bizkaia, Spain

*Corresponding author. Email: cjfernandez@dca.upv.es
2.1. Abstract

Feeding systems for dairy ruminants need to ensure high intake of energy to achieve maximum milk production potential. This might be accomplished by raising the dietary concentration of cereal grain. Increasing the concentration of starch in diets can lead to undesirable ruminal fermentation, and to prevent it, the partial replacement of cereal grain with low starch by-product feeds is recommended. The purpose of the present study was to compare the effect of fed two mixed diets to dairy goats differing in the type of carbohydrate (starch vs. easily degradable fiber). Energy and nitrogen balance, short chain fatty acids in rumen liquor and milk performance in dairy goats during mid lactation were determined. Enteric methane (CH$_4$) emissions and CH$_4$ production from manure were determined as well. Ten multiparous Muciano-Granadina goats were assigned to two isoenergetic and isoproteic diets (19.1 MJ/kg dry matter (DM) and 18.1% of CP, DM basis) in a crossover design. One group was fed a mixed ration with 21.9% of starch (HS diet) and the other (LS diet) with 7.0% of starch. HS diet had 36% of barley (as source of starch) and it was replaced with soy hulls and corn gluten feed in LS diet (as potentially digestible fiber). No differences were observed for dry matter intake in both diets (2.05 kg/d, on average). A significant increase of ruminal acetic acid was found for low starch diet (66.4 and 56.6 mol/100 mol for LS and HS diet, respectively). No significant effect was found among diets for enteric CH$_4$ emissions (28.5 g/d, on average). Manure derived maximum potential yield was (Bo) higher in HS diet, with 5.9 L CH$_4$/kg OM vs. 0.28 L CH$_4$/kg OM for LS diet, probably associated with the low ADF digestibility. Differences among diets were found for milk production (2.4 vs. 2.2 kg/d for HS and LS, respectively), and greater milk fat was observed with LS diet compared with HS (6.4% vs. 5.5%, respectively).

Keywords: Goats, starch, enteric methane, manure CH$_4$ potential production.
EXPERIMENT II: Replacement of cereal with low starch fibrous by-products

2.2. Introduction

Feeding systems for dairy ruminants need to ensure high intake of energy, among other factors, to achieve maximum milk production potential. This might be accomplished by raising the dietary concentration of rapidly degraded non-fibrous carbohydrates, such as starch from cereal grain. Increasing the concentration of starch in diets for dairy cows, however, can lead to undesirable ruminal fermentation, compromising the nutrient supply for production of milk and milk components. To prevent ruminal upsets and health problems, the NRC (2001) recommended the partial replacement of cereal grain with low starch by-product feeds.

Increasing fibrous by-products in the diets generally results in considerably higher enteric CH$_4$ formation compared with starch based diets (Beauchemin et al., 2009). The review of Gerber et al. (2013) concluded that the inclusion of fibrous by-products in the diet of ruminants would likely increase enteric CH$_4$, particularly when inclusion was above 35% to 40% of dry matter intake. Feed ingredients provide the substrates for microbial fermentation, and differences in feed digestibility and chemical composition alter the amount of energy extracted by the microbes and the pattern of volatile fatty acids and CH$_4$ produced (Hindrichsen et al., 2006).

Animal diet can also have a significant impact on manure composition and subsequent gaseous losses (Kreuzer and Hindrichsen, 2006). Modifying proportions of carbohydrates in the diet may lead to different feces composition, which, in turn, will result in different greenhouse gas emissions. Nevertheless, there is scarce information on the effect of replacing carbohydrate sources on ruminant diets over CH$_4$ emissions from their manure (Aguerre et al., 2012; Mathot et al., 2012; Orrico et al., 2012).

The purpose of the present investigation was to compare the effect of fed two mixed diets to dairy goats differing in the type of carbohydrate (starch vs. potentially digestible fiber) in relation to 1) energy, nitrogen (N) balance, and milk performance, and 2) enteric and manure CH$_4$ productions.
2.3. Materials and methods

2.3.1. Animals and feeding

The experimental procedure was approved by the Animal Use and Care Committee of the Polytechnic University of Valencia (Spain) and followed the codes of practice for animals used in experimental works proposed by the European Union (2003). Ten multiparous mature Murciano-Granadina goats of similar body weight (43.01 ± 1.7 kg BW, mean and standard deviation, respectively), 625 ± 4.7 kg in 210 days of lactation, 4th lactation, in mid lactation (106 days in milk) were randomly split into two groups. Nutrient requirements followed the recommendation of Lachica and Aguilera (2003) and Calsamiglia et al. (2009) for goats during lactation. Experimental design was in a cross over (2 treatments × 2 periods) and goats were fed two different mixed rations; one group was fed a mixed ration with 364 g/kg dry matter (DM) of barley grain (high starch diet, HS) and the other diet substituted with barley by 446 g/kg DM of by-products (low starch diet, LS) in the following proportion: 271 g/kg DM soy hulls and 175 g/kg DM gluten feed. The starch levels were 21.9% and 7.0% (both on DM basis) for HS and LS diet, respectively. The goats were fed ad libitum and half the daily ration was offered at 0800 h and half at 1600 h, respectively. Goats had free access to water. Alfalfa hay was cut into 2.5 cm pieces (Cutter SkioldSaby A/S, Kjeldgaardsvej Road, DK 9300 Denmark), and the concentrate was mixed and pelleted along with the premix (Table 2.1). Chemical composition showed a whole mixed ration (forage and pelleted diet). Mixed rations were isoenergetic, with an average value of 19.1 MJ/kg DM for gross energy (GE), and isoproteic 18.1% (DM basis) of CP. Body weight at the beginning and end of the period was determined. The goats were milked once daily at 0700 h with a portable milking machine (Flaco, model DL-170, J. Delgado S.A., Ciudad Real, Spain).

2.3.2. Experimental schedule and measurements

Goats were fed experimental diets on pens during 10 days, then they were allocated in individual metabolism cages at thermoneutrality for another 10 days (20°C - 23°C). Total tract apparent digestibility, energy and N balance, and milk
EXPERIMENT II: Replacement of cereal with low starch fibrous by-products

performance were determined for each animal during a period of 5 consecutive days (five goats per treatment). Feed intake and refusal, and fecal, urine and milk outputs were recorded daily for each goat. Total feces collection were collected in wire-screen baskets placed under the floor of the metabolism crates, and total urine collection was collected through a funnel into plastic buckets containing 100 mL of 10% (vol/vol) H₂SO₄. Representative samples (20%) of diet, feces and urine were stored at −20°C, and pooled for chemical analysis. Immediately after milking, the individual milk yield was weighted and, after mixing, a sample of 10% was put in a bottle with 20 mg of potassium dichromate as a preservative and stored at 4°C before analyses. Ruminal fluid samples were collected by stomach tube before the morning feeding on the last day of the apparent digestibility trial. Ruminal fluid pH was immediately determined using a Model 265A portable pH meter (Orion Research Inc., Beverly, MA, USA). A 5 mL subsample of strained ruminal fluid was acidified to pH 2.0 and mixed with 50% H₂SO₄ and frozen until analysis for volatile fatty acids (VFA).

Gas exchange was measured for each goat during 24 h by a head hood designed for small ruminants. We have one head hood and it was placed in an isolate room without contact with others goats or rest of herd. The head hood dimensions were 36 cm deep × 53 cm wide × 116 cm high, giving a total internal volume of 219 L. The hood was fitted with a polycarbonate window and drawer at the front to facilitate feeding and watering. A tightly woven nylon curtain with a hole for the animal neck, which was attached to the rear panel of the hood, was tied around the animal neck with a nylon drawstring to minimize gas leakage. Fresh outdoor air was introduced into the hood via a hose connected to a box entrance. The gas outlet was across a pipe attached on top of the hood equipped with an air filter to prevent dust in the circuit. Through this pipe the gas flowing from the ventilated head hood to the open-circuit respiratory system, which monitored gaseous exchanges by each animal.

Total airflow through the system was measured by a mass flow meter (Thermal Mass Flowmeter Sensyflow VT-S, ABB, Alzenau, Germany). Air suction was by a centrifugal fan (CST60 Soler Palau Inc., Parets del Vallès, Barcelona,
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Spain) located at the end of the main sampling line with free escape for the air. The gas analyzer (Easyflow 3020 model, ABB, Alzenau, Germany) was calibrated with reference gases. Description of the mobile open-circuit respirometry system used for these measurements was shown in Fernández et al. (2012) and Fernández et al. (2015).

The whole system was calibrated injecting pure N₂, CO₂ and CH₄ into the head box (McLean and Tobin, 1987) determined gravimetrically using a precision scale. Calibration factors were calculated according to Brockway et al. (1971). The average value for the calibration factor was 1.0056 ± 0.00158, 0.9924 ± 0.00915 and 0.9321 ± 0.0053 for O₂, CO₂ and CH₄, respectively. The CH₄ and CO₂ production and O₂ consumption were calculated as described Aguilera and Prieto (1986). An initial atmospheric air sample was collected previous to start the gas exchange measurements with animals and, the gas concentrations were used as reference for calculations.

2.3.3. Feces incubation experiment

Feces were mixed per dietary treatment and stored at 4°C until measurement of gas emissions. Dry matter and organic matter (OM) content of HS and LS-derived feces were analyzed before and at the end of the incubation period.

Methane production was determined in a batch experiment. The experiments were performed in 280 ml bottles incubated at 38°C, controlled by a thermostat (Selecta, Termotronic) with 3 replicates for each treatment. According to Vedrenne et al. (2008), CH₄ production was maximized by diluting samples (1:9 ratio) to avoid any inhibition by excessive NH₃-N concentration. Nitrogen content in the samples was 2% (DM), allowing a headspace ratio equal to 1.6 (manure diluted/headspace). After filling with fecal sample, bottles were sealed with butyl rubber stoppers and the headspace was flushed with N₂ gas during 1 minute.

During incubation, the volume of gas produced was calculated by measuring pressure in the headspace using a manometer (Delta Ohm HD 9220,
absolute pressure meter, 0 - 2000 mbar) and a gas sample was collected in a 9 mL GC vial. After gaseous sample collection, overpressure was removed to restore the atmospheric pressure. Incubation lasted 42 days and measurements were taken in four occasions (days 14, 21, 35 and 42 since start of incubations).

The concentration of CH₄ in biogas was determined by gas chromatography. An Agilent gas chromatograph, model 7890A coupled to a flame ionization detector (FID) and a HT3 Teledyne Tekmar headspace autosampler (HS) was used to perform the analysis. The GC column used was an Alltech (30 m × 0.32 mm × 0 μm film thickness) capillary column and the oven temperature program was 50°C for 3 min, then raised to 75°C at 6°C and held for 5 min. The FID temperature was 250°C and helium was used as the carrier gas (24 psi).

2.3.4. Chemical analyses

Feed, feed refusal and feces samples were dried in a forced air oven at 55°C for 48 h and then ground to pass a 1 mm screen. Urine was dried by lyophilization. Chemical analyses of the diet, feed refusals and feces were conducted for DM, ash, ether extract (EE) and crude protein (CP) according to AOAC (2000). DM of diets and feces was determined by oven-drying at 102°C ± 2°C for 24 h and OM was determined by incineration in an electric muffle furnace at 550°C for 6 h. EE was extracted with petroleum ether after acid hydrolysis to recover saponified fat (Soxtec System HT Tecator, Hillerød, Denmark; 1047 Hydrolyzing Unit and 1043 Extraction Unit). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured in an ANKOM Fiber Analyzer (A220, ANKOM Technologies, Fairport, NY, USA) according to Van Soest et al. (1991) using sodium sulphite and alpha amylase. Non fibrous carbohydrates (NFC) content of diets was calculated by difference method based on chemical analysis of individual feeds as NRC (2001) shown: NFC = 100 − NDF − ash − CP − EE. Gross energy content of the dried samples (feed, feces, urine and milk) was analyzed in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK). The N was analyzed by the Dumas principle (TruSpec CN; LECO Corporation, St. Joseph, MI,
EXPERIMENT I: Replacement of cereal with low starch fibrous by-products

USA). Multiplying N by a factor of 6.25 converted the results to CP. Milk composition (fat, protein, lactose, and total milk solids content) was analyzed with infrared analyzer (MilkoScan FT120 Foss Electric, Hillerød, Denmark). Starch content was determined by enzymatic method (alpha amylase obtained from Sigma-Aldrich, Steinheim, Germany) according to Batey (1982).

Determination of ruminal VFA was based on the method described by Jouany (1982). One μl from each sample was injected in a gas chromatograph (Fisons 8000 series, Milan, Italy) equipped with a split/splitless injector and FID detector. The separation of VFA was made in a DB-FFAP capillary column (30 m × 0.25 mm × 0.25 μm of film thickness) J&W Scientific (USA).

2.3.5. Calculations

The ME intake (MEI) was calculated as the difference between GE intake and energy losses in feces, urine and CH4 (with an energy equivalent value of 39.5 kJ/l CH4, Brouwer, 1965). The heat production (HP) was calculated according to Brouwer (1965) for O2 consumption (l/d), CO2 and CH4 production (l/d) and urine-N (Nur, g/d) as:

\[
HP (kJ) = (16.18 \times O_2) + (5.02 \times CO_2) - (2.17 \times CH_4) - (5.99 \times Nur)
\]

The retained energy (RE) was calculated as the difference between MEI and HP. The body tissue energy (TEbody) was the difference between RE and milk energy (Emilk). The N balance is based on the measurements on nitrogen in feed, feces, urine and milk.

2.3.6. Statistical analyses

The effects of type of diet on intake, digestibility, energy and N balance as well as milk yield and enteric CH4 emissions were analyzed using the mixed model (proc MIXED) from SAS software (2001). The experiment was conducted as a crossover design: each goat received both treatments in 2 periods. The model for the dependent variables included the fixed effect of diet and period with goat as random effect. The following statistical model was used: \( Y = \mu + D + T + \)
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goat + DxT + ε, where Y is the dependent variable, μ is the overall mean, D and T are the fixed effects of diet and period of time, DxT their interaction, goat is the random effect of goat and ε is the random error. Least square means are reported throughout and differences were considered significant at P < 0.05.

The accumulated amount of biogas and CH₄ emissions was calculated by the following equations:

\[ V_{CH_4(i)} = \frac{(nRT)}{P} \] (E1)

\[ Bo = \sum V_{CH_4(i)}/OM_{slurry} \] (E2)

where \( n \) is the CH₄ mol amount in headspace, \( R \) is the ideal gas constant (atm L⋅K\(^{-1}\)⋅mol\(^{-1}\)), \( T \) is the incubating temperature in Kelvin (K), \( P \) is the pressure at the current measurement (atm), \( V_{CH_4(i)} \) is the volume of CH₄ in biogas at the current measurement (L), \( OM_{slurry} \) is the total amount of organic matter in dry weight basis in stored slurry (kg).

CH₄ production data from each treatment were analyzed as repeated measurements during the study. Significant differences are expressed at P < 0.05, unless otherwise stated.

2.4. Results

Effect of period of time and their interaction were not significant, so there are not shown in Tables.

2.4.1. Feed intake, digestibility and performance of goats

Feed intake and total tract apparent digestibility of nutrients by Murciano-Granadina dairy goats are shown in Table 2.2. Dry matter intake was similar on both diets. Ether extract and ADF digestibility were higher (P < 0.05) with low starch diet compared with high starch diet. Total apparent tract digestibility was greater (P < 0.05) in DM, OM, CP and GE for HS diet. The CP digestibility was greater (P < 0.05) for HS diet (78%) than LS (76%). The NDF digestibility was similar in both diets. Differences were found in ADF digestibility, with greater values (P <
EXPERIMENT II: Replacement of cereal with low starch fibrous by-products

0.05) for LS than HS diet (41% vs. 19%, respectively). Starch was completely digested.

Milk yield was higher \((P < 0.05)\) for HS than LS diet (2.4 vs. 2.2 kg/d, respectively), as shown in Table 2.3. Milk dry matter and fat content were different \((P < 0.05)\) between the two diets with greater values for LS compared with HS (15.8 vs. 14.9% for dry matter and 6.4 and 5.5% for fat content).

Rumen fermentation parameters obtained are shown in Table 2.4. No differences were found for pH (average of 6.9) and differences \((P < 0.05)\) were found for ammonia N production (18.2 vs. 25.4 mg/dl in HS and LS diet, respectively). No differences were found for total VFA, although differences \((P < 0.05)\) were found in most of the VFA studied. A significant increase of acetic, butyric and N-caproic acids in the rumen was observed in LS diet. The rest of VFA showed differences \((P < 0.05)\) between diets with higher values for HS diet.

2.4.2. Metabolic energy partition and nitrogen balance

Daily energy balance is displayed in Table 2.5. No significant differences were observed for GE intake (39.1 MJ/d, on average) and higher \((P < 0.05)\) energy losses in feces \((E_{\text{feces}})\) were found for LS than HS (13.3 vs. 12.0 MJ/d, respectively). The HP was different \((P < 0.05)\) between HS and LS treatment, with greater values for LS compared with HS (13.6 vs. 12.9 MJ/d, respectively). With regard to the rest of the energy balance, no differences were found and positive energy balance was observed between treatments.

The daily N balance is displayed in Table 2.6. Differences \((P < 0.05)\) were detected for urine-N in LS related with HS (26.8 vs. 21.7 g/d, respectively) and the recovered of N was higher \((P < 0.05)\) for HS than LS (10.9 vs. 4.4 g/d, respectively).

Table 2.7 shows enteric CH\(_4\) emissions from goats. The average CH\(_4\) emissions from the goat’s digestive tracts (enteric fermentation) were similar between diets and averaged at 28.5 g/goat per day. Higher values \((P < 0.05)\) with LS than HS diet in CH\(_4\) emitted per kg of milk (13.2 vs. 11.7 g/kg, respectively) were found.
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2.4.3. Manure CH\textsubscript{4} production

Goat manure characteristics before incubation are presented in Table 2.8. Higher OM in feces ($P < 0.05$) was found with LS than HS, and lower ($P < 0.05$) ADF was found with LS compared with HS diet (0.43 vs. 0.52 g/kg DM feces, respectively). Cumulative CH\textsubscript{4} production of goat feces as function of time during 42 days incubation is shown in Figure 1. The CH\textsubscript{4} production from HS (5.9 L CH\textsubscript{4}/kg OM) treatment was higher ($P < 0.05$) compared to LS treatment (0.28 L CH\textsubscript{4}/kg OM). The peak of CH\textsubscript{4} production in HS treatment took place 20 days after incubation started. For LS treatment, CH\textsubscript{4} production was negligible during the experiment.

2.5. Discussion

2.5.1. Effect of level of starch on intake, digestibility, rumen parameters and performance in dairy goats

The higher content of barley and lower ADF content in HS diet than LS diet appeared to be the main factor responsible for the lower DM, OM and CP apparent digestibility found in LS diet. According to NRC (2001), soy hulls and corn gluten feed are two by-products feeds that are highly digestible and are low in non fibrous carbohydrates. Therefore, differences in digestibility depend on type and dietary proportions of different carbohydrates such as, cellulose, hemicellulose, pectin, starch, etc.

Total starch digestibility was found in both diets. Regarding fiber digestibility, lower ADF digestibility was observed for HS diet compared with LS, and it could be important regarding fecal fermentation. Non fibrous carbohydrates constitute an important energy rich fraction of ruminant diets, which consists mostly of starch, pectin, galactans and simple sugars. Non fibrous carbohydrates usually have a fast fermentation rate and stimulate the production of propionate. In our study, HS diet has greater values of both NFC (28.7% and 21.8%, for HS and LS diet, respectively) and propionic acid (21.4 and 18.0 mol/100 mol for HS and LS diet, respectively) than LS diet (higher in acetic acid). The propionic acid concentration was higher on HS than LS which indicated that goats had
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more precursors available for gluconeogenesis (Van Knegsel et al., 2007), which could increase the amount of precursors available for lactose syntheses and thereby milk production. Therefore, these higher NFC and starch in HS diet seem to be the responsible of the lower ADF digestibility, minor ammonia N in the rumen, more excretion of N in urine and lower fat content in milk.

Milk yield was higher \( P < 0.05 \) for HS than LS diet (2.4 and 2.2 kg/d, respectively), as is shown in Table 2.3. Milk fat content was greater for LS than HS (6.4 and 5.5%, respectively) as it had greater fiber and fat content, superior ADF digestibility and, higher acetic acid production than HS. The depression in milk fat upon feeding starch rich diets has been explained by a shift from a high availability of fat precursors to glucose and by a shift from lipogenesis to gluconeogenesis (Van Knegsel et al., 2007). In our study, no differences were found for milk protein and lactose contents between diets (3.9% and 4.7% respectively, on average).

2.5.2. Effect of level of starch on metabolic energy utilization and nitrogen balance of the goats

The average GE intake was 39.1 MJ/d. The MEI was the same for the two diets (23.9 MJ/d, on average) and the HP was different between diets, with greater value for the diet higher in fiber (13.6 vs. 12.9 MJ/din LS and HS, respectively). Lipogenic nutrients, which increase milk fat yield (Van Knegsel et al., 2007), increase the partitioning of ME into milk and consequently decrease the partitioning of ME into body reserves. The \( E_{\text{milk}} \) content was the same for the two diets (9.0 MJ/d, on average). We did not find differences between diets for tissue energy deposition (1.6 MJ/d, on average) and, greater efficiency of ME use for lactation (38%) than retention (7%) was observed in both diets.

More energy losses in feces were found for LS than HS (13.3 vs. 12.0 MJ/d, respectively), probably due to goat excreted more feces on LS than HS (Table 2.8). The \( E_{\text{urine}} \) was 1 MJ/d on average for the two diets and, higher excretion of N in urine was found for LS diet. These observations are in agreement with the diet rich in fibrous ingredients (LS) alongside the larger ammonia N found (Table
EXPERIMENT II: Replacement of cereal with low starch fibrous by-products

2.4). Goats fed LS diet contain greater fibrous by-products and superior ammonia N production ($P < 0.05$) than HS diet (25.4 vs. 18.2 mg/dl, respectively). This difference was probably linked to the lower N expenditure by ruminal bacteria to synthesize microbial protein in LS diet (Casper et al., 1999). The $N_{\text{urine}}$ losses were greater in LS compared with HS (26.8 vs. 21.7 g/d, respectively) likely due to poor efficiency for protein use (greater values of ammonia N on ruminal liquor in LS than HS, Table 2.4.). When carbohydrate availability increases, ammonia production decreases because of a direct incorporation of ammonia N into microbial protein. Ruminal ammonia N not utilized for microbial protein synthesis is excreted in urine (Hoover ans Stokes, 1991). Diet higher in starch seems to increase the amount of fat synthesis in the body (Corbett and Freer, 2003) and therefore energy retention. In our study no differences in energy retention were found between diets.

2.5.3. Effect of starch and potentially digestible fiber on enteric methane emissions and manure CH$_4$ production

Energy losses in CH$_4$ were not significantly different among treatments, with an average value of 1.6 MJ/d (Table 2.5). The CH$_4$ values obtained by other authors for lactating goats are variable, so Aguilera et al. (1990) found values that range from 1.2 to 1.9 MJ/d, both with diets based on pelleted alfalfa hay and barley. Average values of 2.6 and 2.3 MJ/d lost as enteric CH$_4$ for forage and non forage diets, respectively, were found by Bava et al. (2001).

The average CH$_4$ emissions from the goat’s digestive tracts (enteric fermentation) was similar between diets; averaged at 28.5 g/goat per day and Ym (methane conversion factor) at 4.1 (Table 2.7). In our study the feed intake during the digestibility was the same that during CH$_4$ emissions measurements. It is often claimed that forage based diets generally results in considerably higher enteric CH$_4$ formation than mixed or concentrated based diets (Johnson and Johnson, 1995). But Gerber et al. (2013) indicate that inclusion of fibrous by-products in the diet of ruminant will increase enteric CH$_4$ particularly when inclusion is above 35% to 40% of DMI, and in our study was lower. The type of fiber
EXPERIMENT II: Replacement of cereal with low starch fibrous by-products

(highly fermentable, such as the by-product used) is very different from that is usually associated to CH$_4$ production (fiber from forages), lack of synchronization of carbohydrates and protein in LS diet (significant higher values on ruminal liquor ammonia N) and the fat level in LS (1% point higher than HS diet) were the responsible of the absence of effect of LS diet on CH$_4$ enteric emissions. High acetic acid productions are accompanied with high levels of available hydrogen and hence with high methane production. However, soluble sugars modify the process. Fiber by-products contain more soluble sugars than roughage. Greater proportion of butyric acid was observed in LS compared with HS, because fiber quality of by-products could be favour fermentation processes to butyric acid (Waldo, 1973).

The DM and OM content in goat feces before incubation was slightly higher than data reported in previous studies for other ruminants (Triolo et al., 2011). The highest CH$_4$ production in HS treatment took place 20 days after incubation started. For LS treatment, CH$_4$ production was negligible during the experiment. There were significantly higher amount of carbohydrates available for fermentation in excreta from HS than from LS, which could have given as result higher amounts of CH$_4$ production from HS diet, as shown in Figure 1. In brief, in our study starch was 100% digested so there was not starch in the feces; however, the digestibility of ADF with HS diet was low compared with LS diet, indicating higher hemicellulose in feces (see Table 2.2).

Reports in the literature on the effects of different types of diets on CH$_4$ emissions from the goat exhaled air or manure are very scarce. Methane emissions from the feces increased shortly after the start of incubation and reached its peak between weeks 2 and 3. Several authors, Hindrichsen et al. (2006) and Vedrenne et al. (2008) observed that with cattle manure the plateau phase during batch digestion was reached later, between weeks 6 and 9. In this study, HS diet with mainly barley as concentrate had low digestibility of ADF and greater residue of ADF in feces. Consequently, manure derived maximum potential yield (B$_0$) was higher in HS diet (5.9 L CH$_4$/kg OM) than LS diet (0.28 L CH$_4$/kg OM). The review of Knapp et al. (2014) denotes that feed ingredients
provide the substrates for microbial fermentation, and differences in feed digestibility and chemical composition alter the amount of CH$_4$ production.

### 2.6. Conclusion

Comparison of starch diet with potentially digestible fiber ingredients was evaluated. Positive energy and N balance were obtained in all goats, independent of the treatment. Lower milk production was found in LS compared with HS (2.2 vs. 2.4 kg/d, respectively). Greater fat content in milk was found in the low starch diet (6.4%) compared with the higher (5.5%), because low starch diet replaced barley with fibrous by-products. Both diets showed identical Ym values (4.1% on average), so the type of fiber used to reduce the level of starch and the higher level of fat added to diet LS (1 point higher than HS) were likely responsible for the lack of effect on CH$_4$ enteric emissions. The CH$_4$ production from manure derived from the rich starch diet was 15 times higher than from the low starch diet after 42 days of incubation, due to greater amount of hemicelluloses in HS feces. Goats at mid lactation could utilize fibrous by-product as cereal replacement, without detrimental effects on energy metabolism, milk performance and CH$_4$ emissions.

### 2.7. Acknowledgements

This study was supported by INIA Project, Spain (ref. RTA2011-00107-C02).

### 2.8. References


Aguilera, J.F., Prieto, C. (1986) Description and function of an open-circuit respiration plant for pigs and small ruminants and the techniques used to
EXPERIMENT II: Replacement of cereal with low starch fibrous by-products

measure energy metabolism. Archiv für Tierernaehrung 36, 1009-1018. doi:10.1080/17450398609429522


EXPERIMENT II: Replacement of cereal with low starch fibrous by-products


EXPERIMENT II: Replacement of cereal with low starch fibrous by-products


EXPERIMENT II: Replacement of cereal with low starch fibrous by-products


EXPERIMENT II: Replacement of cereal with low starch fibrous by-products

Table 2.1. Ingredients (g/kg DM) and chemical composition of diets (% of DM): high starch (HS) and low starch (LS) diets.

<table>
<thead>
<tr>
<th>Ingredients, g/kg DM</th>
<th>HS</th>
<th>LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa hay</td>
<td>413</td>
<td>415</td>
</tr>
<tr>
<td>Barley</td>
<td>364</td>
<td>-</td>
</tr>
<tr>
<td>Soy meal 44% crude protein</td>
<td>155</td>
<td>100</td>
</tr>
<tr>
<td>Soy hulls</td>
<td>-</td>
<td>271</td>
</tr>
<tr>
<td>Gluten feed 18% crude protein</td>
<td>-</td>
<td>175</td>
</tr>
<tr>
<td>Lard(^b)</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>By-pass fat(^c)</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Beet molasses</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Bicalcium phosphate</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Premix(^d)</td>
<td>2.6</td>
<td>2.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical composition, %</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>87.5</td>
<td>88.3</td>
</tr>
<tr>
<td>Organic matter</td>
<td>91.8</td>
<td>91.5</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>18.2</td>
<td>18.0</td>
</tr>
<tr>
<td>Ether extract</td>
<td>4.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>40.6</td>
<td>46.5</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>19.1</td>
<td>27.0</td>
</tr>
<tr>
<td>NFC(^e)</td>
<td>28.7</td>
<td>21.8</td>
</tr>
<tr>
<td>Starch</td>
<td>21.9</td>
<td>7.0</td>
</tr>
<tr>
<td>Gross energy, MJ/kg DM</td>
<td>19.0</td>
<td>19.2</td>
</tr>
</tbody>
</table>

\(^a\)HS = high starch diet; LS = low starch diet; DM = dry matter.

\(^b\)Fused lard provided by VALGESS S.L., Carpesa, Valencia, Spain.

\(^c\)By-pass fat of palm fatty acid distillate. Provided by Norel Animal Nutrition, Norel S.A., Spain.

\(^d\)Premix provided by NACOOP S.A. España. (ppm or UI per kilogram of premix): Se, 40; I, 250; Co, 80; Cu, 3000; Fe, 6000; Zn, 23400; Mn, 29000; S, 60000; Mg, 60000; vitamin A, 2000000 UI; vitamin D3, 400000; vitamin E, 2000 ppm; nicotinic acid, 10000; choline, 20300.

\(^e\)NFC = 100 - (neutral detergent fiber + crude protein + ash + ether extract); non fibrous carbohydrate.
**EXPERIMENT II:** Replacement of cereal with low starch fibrous by-products

Table 2.2. Daily intake, total tract digestibility and performance in lactating goats (n = 20).

<table>
<thead>
<tr>
<th>Items(^{a})</th>
<th>HS</th>
<th>LS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>2.03</td>
<td>2.07</td>
<td>0.040</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Digestibility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>68</td>
<td>65</td>
<td>0.6</td>
<td>0.01</td>
</tr>
<tr>
<td>OM, %</td>
<td>70</td>
<td>66</td>
<td>0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>CP, %</td>
<td>78</td>
<td>76</td>
<td>0.8</td>
<td>0.02</td>
</tr>
<tr>
<td>EE, %</td>
<td>84</td>
<td>86</td>
<td>0.5</td>
<td>0.07</td>
</tr>
<tr>
<td>NDF, %</td>
<td>47</td>
<td>48</td>
<td>0.8</td>
<td>NS</td>
</tr>
<tr>
<td>ADF, %</td>
<td>19</td>
<td>41</td>
<td>2.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Starch, %</td>
<td>100</td>
<td>100</td>
<td>0.1</td>
<td>NS</td>
</tr>
<tr>
<td>GE, %</td>
<td>69</td>
<td>67</td>
<td>0.7</td>
<td>0.03</td>
</tr>
</tbody>
</table>

HS = high starch diet; LS, low starch diet; SEM = standard error of the mean.

\(^{a}\)DMI = dry matter intake; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; GE = gross energy.
**Table 2.3. Milk performance in lactating goats (n = 20).**

<table>
<thead>
<tr>
<th>Items</th>
<th>HS</th>
<th>LS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield, kg/goat/day</td>
<td>2.4</td>
<td>2.2</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>DM, %</td>
<td>14.9</td>
<td>15.8</td>
<td>0.23</td>
<td>0.01</td>
</tr>
<tr>
<td>Fat, %</td>
<td>5.5</td>
<td>6.4</td>
<td>0.17</td>
<td>0.01</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.9</td>
<td>3.9</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.7</td>
<td>4.7</td>
<td>0.06</td>
<td>NS</td>
</tr>
</tbody>
</table>

HS = high starch diet; LS, low starch diet; SEM = standard error of the mean.

^DM = dry matter.
Table 2.4. Effect of diet on ruminal pH, ammonia N (NH$_3$ N, mg/dl) and volatile fatty acids (VFA, mol/100 mol) in lactating goats (n = 20).

<table>
<thead>
<tr>
<th>Items$^\text{A}$</th>
<th>HS</th>
<th>LS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.8</td>
<td>6.9</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>NH$_3$ N</td>
<td>18.2</td>
<td>25.4</td>
<td>4.37</td>
<td>0.05</td>
</tr>
<tr>
<td>Total VFA, meq/l</td>
<td>26.6</td>
<td>24.8</td>
<td>0.76</td>
<td>NS</td>
</tr>
<tr>
<td>Acetic</td>
<td>56.6</td>
<td>66.4</td>
<td>0.69</td>
<td>0.01</td>
</tr>
<tr>
<td>Propionic</td>
<td>21.4</td>
<td>18.0</td>
<td>0.93</td>
<td>0.01</td>
</tr>
<tr>
<td>Isobutyric</td>
<td>4.0</td>
<td>2.5</td>
<td>0.17</td>
<td>0.03</td>
</tr>
<tr>
<td>Butyric</td>
<td>8.5</td>
<td>12.9</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>5.6</td>
<td>3.2</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>N-valeric</td>
<td>3.3</td>
<td>1.5</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>N-caproic</td>
<td>0.3</td>
<td>0.4</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

HS = high starch diet; LS = low starch diet; SEM = standard error of the mean.

$^\text{A}$NH$_3$ N = ammonia nitrogen; VFA = volatile fatty acids; N = nitrogen.
Table 2.5. Energy balance in lactating goats (n = 20).

<table>
<thead>
<tr>
<th>Items^</th>
<th>HS</th>
<th>LS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEI</td>
<td>38.4</td>
<td>39.8</td>
<td>0.76</td>
<td>NS</td>
</tr>
<tr>
<td>Efeces</td>
<td>12.0</td>
<td>13.3</td>
<td>0.45</td>
<td>0.02</td>
</tr>
<tr>
<td>DEI</td>
<td>26.4</td>
<td>26.5</td>
<td>0.44</td>
<td>NS</td>
</tr>
<tr>
<td>Eurine</td>
<td>1.2</td>
<td>0.8</td>
<td>0.16</td>
<td>NS</td>
</tr>
<tr>
<td>E_methane</td>
<td>1.6</td>
<td>1.6</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>MEI</td>
<td>23.7</td>
<td>24.1</td>
<td>0.40</td>
<td>NS</td>
</tr>
<tr>
<td>HP</td>
<td>12.9</td>
<td>13.6</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Emilk</td>
<td>9.1</td>
<td>8.9</td>
<td>0.24</td>
<td>NS</td>
</tr>
<tr>
<td>TE_body</td>
<td>1.7</td>
<td>1.5</td>
<td>0.41</td>
<td>NS</td>
</tr>
</tbody>
</table>

HS = high starch diet; LS = low starch diet; SEM = standard error of the mean.

^GEI = gross energy intake; E = energy; DEI = digestible energy intake; MEI = metabolizable energy intake; HP = heat production; TE = tissue energy.
Table 2.6. Nitrogen balance in lactating goats (n = 20).

<table>
<thead>
<tr>
<th>Items(^a)</th>
<th>HS</th>
<th>LS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen balance, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N(_{\text{intake}})</td>
<td>61.1</td>
<td>59.6</td>
<td>1.19</td>
<td>NS</td>
</tr>
<tr>
<td>N(_{\text{feces}})</td>
<td>14.3</td>
<td>15.2</td>
<td>0.57</td>
<td>NS</td>
</tr>
<tr>
<td>N(_{\text{urine}})</td>
<td>21.7</td>
<td>26.8</td>
<td>0.91</td>
<td>0.01</td>
</tr>
<tr>
<td>N(_{\text{milk}})</td>
<td>14.2</td>
<td>13.2</td>
<td>0.32</td>
<td>NS</td>
</tr>
<tr>
<td>N recover</td>
<td>10.9</td>
<td>4.4</td>
<td>1.28</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(\text{HS} = \text{high starch diet; LS = low starch diet; SEM = standard error of the mean.}\)

\(^a\text{N} = \text{nitrogen.}\)
Table 2.7. Enteric methane formation in lactating goats (n = 20).

<table>
<thead>
<tr>
<th>Items^</th>
<th>HS</th>
<th>LS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₄, g/d</td>
<td>28.4</td>
<td>28.5</td>
<td>0.54</td>
<td>NS</td>
</tr>
<tr>
<td>CH₄/DMI, g/kg</td>
<td>14.1</td>
<td>13.8</td>
<td>0.36</td>
<td>NS</td>
</tr>
<tr>
<td>CH₄/OM intake, g/kg</td>
<td>15.4</td>
<td>15.1</td>
<td>0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Ym, (CH₄/GEi)</td>
<td>4.2</td>
<td>4.0</td>
<td>0.11</td>
<td>NS</td>
</tr>
<tr>
<td>CH₄/milk, g/kg</td>
<td>11.7</td>
<td>13.2</td>
<td>0.42</td>
<td>0.03</td>
</tr>
</tbody>
</table>

HS = high starch diet; LS = low starch diet; SEM = standard error of the mean.
^CH₄ = methane; DMI = dry matter intake; OM = organic matter; Ym = methane energy/gross energy intake; GEi = gross energy intake.
EXPERIMENT II: Replacement of cereal with low starch fibrous by-products

Table 2.8. Chemical composition of the feces previous to incubation (g/kg DM feces).

<table>
<thead>
<tr>
<th>Items^</th>
<th>HS</th>
<th>LS</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>0.20</td>
<td>0.25</td>
<td>0.011</td>
<td>0.02</td>
</tr>
<tr>
<td>CP</td>
<td>0.14</td>
<td>0.18</td>
<td>0.011</td>
<td>0.08</td>
</tr>
<tr>
<td>EE</td>
<td>0.10</td>
<td>0.11</td>
<td>0.006</td>
<td>NS</td>
</tr>
<tr>
<td>NDF</td>
<td>0.35</td>
<td>0.39</td>
<td>0.016</td>
<td>NS</td>
</tr>
<tr>
<td>ADF</td>
<td>0.52</td>
<td>0.43</td>
<td>0.021</td>
<td>0.02</td>
</tr>
<tr>
<td>Starch feces, mg/kg DM feces</td>
<td>0.50</td>
<td>0.20</td>
<td>0.010</td>
<td>NS</td>
</tr>
</tbody>
</table>

HS = high starch diet; LS = low starch diet; SEM = standard error of the mean.
^OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; DM = dry matter.
**EXPERIMENT II**: Replacement of cereal with low starch fibrous by-products

Figure 2.1. Cumulative methane production $B_o$ (L CH$_4$/kg OM) from LS and HS diets in diluted goat feces during 42 days of incubation.

$H_S$ = high starch diet; $L_S$ = low starch diet; $B_o$ = cumulative methane production; OM = organic matter.
EXPERIMENT III: Murciano-Granadina goat performance after replacing barley grain with fibrous by-products
EXPERIMENT III: Replacement of barley grain with fibrous by-products

Murciano-Granadina goat performance after replacing barley grain with fibrous by-products

C. Ibáñez¹, P. Criscioni² and C. Fernández²*

²Research Center ACUMA. Animal Science Department. Polytechnic University of Valencia. 46022 Valencia, Spain.
*Correspondence: Carlos Fernández. ACUMA Research Center. Polytechnic University of Valencia. 46022-Valencia-Spain (email: cjfernandez@dca.upv.es). Tel: +00 34 963 877 007
EXPERIMENT III: Replacement of barley grain with fibrous by-products

3.1. Abstract

The objective of this experiment was to study the effects of substitution of dietary barley grain by orange pulp or soybean hulls on energy, nitrogen and carbon balance, methane emission and milk performance in dairy goats. Twelve Murciano-Granadina dairy goats in mid lactation were selected and divided into three groups based on similar body weight (42.1 ± 1.2 kg) and milk yield (2155.2 ± 60.3 g/goat/day). The experiment was conducted in an incomplete crossover design where one group of four goats was fed a mixed ration of barley grain (BRL), another group of four goats replaced barley grain with orange pulp (OP) and the last group of four goats with soybean hulls (SH). The goats were allocated to individual metabolism cages. After 14 days of adaptation, feed intake, total fecal and urine output and milk yield were recorded daily over a 5 days period. Then, gas exchange measurements were recorded by a mobile open-circuit indirect calorimetry system using a head box. Dry matter intake was similar for all three groups (2.03 kg/d, on average). No influence of the diet was observed for MEI, HP and milk energy (1352.4, 822.8 and 505.3 kJ/kg of BW\(^{0.75}\) on average, respectively) and the efficiency of use of ME for milk production was 0.61. The OP and SH diets showed greater \((P < 0.05)\) fat mobilization (-42.8 kJ/kg of BW\(^{0.75}\), on average) than BRL (19.2 kJ/kg of BW\(^{0.75}\)). Pentadecanoic acid and heptadecanoic acid are potential biomarkers of rumen function and the higher contents found in the milk of OP and SH goats than BRL suggest a negative impact of these diets on rumen bacterial metabolism. Replacement of cereal grain with fibrous by-products did not increased enteric methane emissions (40.7 g/goat per day, on average). Therefore, lactating goats could utilize dry orange pulp and soybean hulls diets without detrimental effect on milk performance.

Keywords: lactating goats, mixed rations, methane emissions, Ym.
3.2. Introduction

To achieve maximum milk production potential by means acceptable to consumers, feeding systems for dairy ruminants need to ensure high intake of energy, among other factors. This might be accomplished by raising the dietary concentration of rapidly degraded non fibrous carbohydrates (NFC), such as starch from cereal grain. Increasing the concentration of NFC in diets for dairy cows, however, can lead to undesirable ruminal fermentation, compromising the nutrient supply for production of milk and milk components. The partial replacement of cereal grain with low starch by-product feeds represents a potential alternative to overcome this limitation. By-products from agriculture may be of interest not only for reducing feeding cost but also to reduce environmental problems associated with side-effect accumulation (Vasta et al., 2008).

Recently, there is increasing interest in by-products as partial substitution of traditional feedstuffs in ruminant feeding. From a nutritional point of view, by-products are included in the ration to supply energy and protein, but are often also characterized by high fiber content. This is the case of orange pulp or soybean hulls that are typically used as grain replacers. A large number of the citrus by-products feedstuffs, including orange pulp, are suitable for inclusion in ruminant diets because of the ability of ruminants to ferment high fiber feeds in the rumen (Grasser et al., 1995). According to FEDNA (2010) the neutral detergent fiber (NDF) level of dry orange pulp is intermediate (25%) between barley grain (17%) and soy hulls (58%), contains relatively large amounts of pectins (25%) and sugars (23%) and low amount of lignin (2%) and starch (0.5%); also very limited amount of available nitrogen (6% of crude protein [CP]). Soybean hull has similar CP content than barley grain (11%), is high in NDF (58%, high in cellulose) but it is low in lignin (2%), NFC (24%), sugars (1.5%) and with no starch content (barley grain has 51% of starch).

In the ruminant nutrition, decreased production of methane (CH$_4$) can represent an improvement in feed efficiency, because ruminants loose between 2-12% of the gross dietary energy in the form of CH$_4$ (Johnson and Johnson, 1995).
Besides, ruminants contribute to global warming through emission of nitrous oxide from urine and feces. To reduce nitrogen (N) excretion and improve N efficiency of ruminant, dietary levels of N and optimal balance between N and energy substrates in the diet should be aimed.

Our hypothesis is that orange pulp and soybean hulls could replace cereal based concentrate in goat’s diets without compromising energy and protein partitioning in lactating goats. The aim of this experiment was to study the effect of substitution of barley grain in the mixed diet by dry orange pulp or soybean hulls on energy partitioning, enteric and manure derived CH₄ emissions, carbon (C) and N balance and milk performance in dairy goats during mid lactation.

3.3. Materials and methods

The experimental procedure was approved by the Animal Use and Care Committee of the Polytechnic University of Valencia (Spain) and followed the codes of practice for animals used in experimental works proposed by the European Union (2003).

3.3.1. Animals and feeding

The experiment was conducted at the Experimental Farm of Animal Science Department (ACUMA Research Center), Valencia (Spain). Twelve multiparous mature Murciano-Granadina dairy goats in mid lactation were selected and divided into three groups based on similar body weight (BW) and milk production. The experiment was conducted in an incomplete crossover design where one group of four goats was fed a mixed ration of barley grain, another group of four goats replaced barley grain with orange pulp and the last group of four goats with soybean hulls. Goats were fed above production level and ingredients and chemical composition of the three formulated diets are shown on Table 3.1. The total amount of feed offered was 2.4 kg per goat and day. Goats were fed mixed diets with 800 g of alfalfa hay per day and 1600 g of concentrate per goat and day (forage and concentrate ratio = 33/67). The concentrate was mixed and pelleted along with the premix. One group was fed
concentrate with 670 g/kg DM of barley (BRL diet). The other two groups substituted barley grain with by-products: dry orange pulp (OP diet) and soybean hulls (SH diet). Requirements of the goats were obtained using the recommended values of AFRC (1993) and FEDNA (2010). Diets were supplemented with a salt vitamin-mineral premix and water was freely available at all times. Chemical composition shown in Table 3.1 is whole mixed ration (forage and pelleted concentrate). The mean gross energy (GE) of the three diets was 18 MJ/kg DM. The main difference among diets was the source of carbohydrates. Starch levels were 33%, 5% and 2% (on DM basis) for BRL, OP and SH diets, respectively. NDF values were 42%, 31% and 55% (on DM basis) for BRL, OP and SH diets, respectively. Mixed diets contained similar amounts of CP (13%, on DM basis). All goats were housed in a building in which the environment was partially controlled (HOBO; BoxCarPro3 software).

3.3.2. Experimental schedule and measurements

Apparent total tract digestibility, gas exchange, energy partitioning, C and N balance, oxidation of nutrients and milk composition and yield were determined. The experiment was conducted in a crossover design in two 30 days periods. During the adaptation, goats were fed the experimental diets in pens for 7 days and then allocated to individual metabolism cages at thermoneutrality (20-23 °C determined by a Hobo probe, ONSET data loggers, Cape Cod, MA, USA) for another 7 days. Next, data on the feed offered and refused and the total fecal, urine and milk output were recorded daily for each goat during a 5 days period, as well as BW at the beginning and end of the period. Feces were collected in wire-screen baskets placed under the floor of the metabolism crates and urine was collected through a funnel into plastic buckets containing 100 mL 10% (vol/vol) of H₂SO₄ to acidify the urine of each goat. The acidification of urine was necessary to prevent microbial degradation and the loss of volatile ammonia-N (NH₃-N). Representative samples (10%) of diet, feces and urine were collected over 5 consecutive days, stored at -20 °C and pooled for chemical analysis. The goats were milked once daily at 0800 h with a portable milking machine (Flaco, model DL-170, J. Delgado S.A., Ciudad Real, Spain).
Immediately after milking, the individual milk yield was measured and a sample of 10% was put in a bottle and frozen until analysis. In addition, samples were collected into plastic vials that contained 20 mg of potassium dichromate as a preservative and taken to the Interprofessional Dairy Laboratory of the Valencia Community Region (LICOVAL, Valencia, Spain) for compositional analysis (dry matter, crude protein, fat and lactose). At the end of the digestibility trial, blood samples were collected from each goat. Jugular blood samples were taken before the morning feeding. Blood was sampled in 10 mL tubes treated with EDTA or Li-heparine, immediately centrifuged for plasma separation and stored at -20°C. Ruminal fluid samples were collected by stomach tube before the morning feeding on the last day of the apparent digestibility trial. Ruminal fluid pH was immediately determined using a Model 265A portable pH meter (Orion Research Inc., Beverly, MA, USA). A ruminal fluid sample was acidified with 50% H$_2$SO$_4$ and frozen until later determination of NH$_3$-N. Samples for analysis of VFA were mixed with H$_3$PO$_4$ and kept frozen until analysis.

Then, gas exchange was measured for each goat during 24 h (6 goats/treatment) by an indirect calorimetric system based on a ventilated head box designed for small ruminants. To this end, 12 days were taken for each period in the incomplete cross over design. The respirometry system has a head hood, a flow meter (Thermal Mass Flowmeter Sensyflow VT-S, ABB, Alzenau, Germany) and air suction provided by a centrifugal fan (CST60 Soler Palau Inc., Parets del Vallès, Barcelona, Spain). The methane (CH$_4$) and carbon dioxide (CO$_2$) concentration were measured using the infrared principle and oxygen (O$_2$) was measured by the paramagnetic principle (Easyflow Gas Analyzer, model 3020, ABB, Alzenau, Germany). Although the unit was an autocalibrated model, the analyzers were calibrated with reference gases before each test. Fernández et al. (2012; 2015) described the mobile open-circuit respirometry system used for these measurements.

The whole system was calibrated by injecting pure N$_2$ and CO$_2$ into the head box (McLean and Tobin, 1987), determined gravimetrically using a precision scale (MOBBA mini-SP 0.2-30 kg, Industrial Weighing System, Barcelona, Spain).
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Calibration factors were calculated according to Brockway et al. (1971). The CH₄ and CO₂ production and O₂ consumption were calculated as described by Aguilera and Prieto (1986). An initial atmospheric air sample was collected and the gas concentrations were used as reference for calculations.

3.3.3. Chemical analyses

Feed, feed refusal and feces samples were first dried in a forced air oven at 55 ºC for 48 h then ground to pass a 1 mm screen before analysis. Urine and milk were dried by lyophillization. Chemical analyses of the diet, refusals and feces were conducted according to AOAC (2000) for DM, ash and ether extract (EE). The DM of diets and feces was determined by oven-drying at 102 ± 2 ºC for 24 h. Ash concentration was measured by incineration in an electric muffle furnace at 550 ºC for 6 h to determine OM. The EE was extracted with petroleum ether after acid hydrolysis to recover saponified fat (Soxtect System HT Tecator, Hillerød, Denmark: 1047 Hydrolyzing Unit and 1043 Extraction Unit). The NDF and ADF were measured in an ANKOM Fiber Analyzer (A220, ANKOM Technologies, Fairport, NY, USA) according to Mertens (2002) and AOAC (2000), respectively. The NDF was determined using sodium sulphite and alpha amylase. The NFC content of diets was calculated by difference method based on chemical analysis of individual feeds according to NRC (2001): NFC = 100 – NDF – ash – CP – EE. The GE content of the dried samples (feed, feces, urine and milk) was analyzed by combustion in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK). Starch content was determined by enzymatic method (α-amylase obtained from Sigma-Aldrich, Steinheim, Germany) according to Batey (1982). The C and N were analyzed by the Dumas principle (TruSpec CN; LECO Corporation, St. Joseph, MI, USA). Multiplying N by a factor of 6.25 converted the results to CP.

Milk composition (fat, protein, lactose, citrate and total milk solids content) was analyzed with an infrared analyzer (MilkoScan FT120 Foss Electric, Hillerød, Denmark). Fatty acid (FA) methyl esters of total milk lipids were prepared directly as previously described O’Fallon et al. (2007). The FA methyl esters were analyzed in a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a
split/splitless injector and a flame ionization detector. Separation of methyl esters was performed in a fused silica capillary column SP™ 2560 (Supelco, PA, USA) (100 m x 0.25 mm x 0.2 µm film thickness). The carrier gas was Helium at a linear velocity of 20 cm/sec. The samples were injected with a split ratio of 1/100. The initial oven temperature was set at 140 ºC held for 5 min and increased to 240 at 4 ºC/min and finally maintained at that temperature for 30 min. Both detector and injector temperatures were set at 260 ºC.

A subset of milk samples was collected and analyzed for milk urea. Urea and total protein were analyzed in urine. In plasma glucose, non esterified fatty acids (NEFA), ß-hydroxybutyrate (BHBA), ketone bodies and triglycerides were also analyzed. All these samples were send to a diagnostic laboratory (Laboratorio de Diagnóstico General, Comte Borrell, 08015 Barcelona, Spain) for determinations.

\[ \text{NH}_3-N \text{ content of ruminal fluid samples was analyzed by the Kjeldahl procedure (2300 Kjeltec Analyzer Unit Foss Tecator, Hillerød, Denmark). Determination of ruminal VFA was based on the method described by Jouany (1982) using a gas chromatograph (Fisons 8000 series; Fisons Instruments SpA, Milan, Italy) equipped with a split/splitless injector and flame ionization detector.} \]

### 3.3.4. Calculations

The ME intake (MEI) was calculated as the difference between GE intake and energy losses in feces, urine and CH₄ (with an energy equivalent value of 39.5 kJ/L CH₄; Brouwer, 1965).

The heat production (HP) was determined from measurements of O₂ consumption, CO₂ and CH₄ production, and urine-N (N\text{urine}), using the equation of Brouwer (1965):

\[ \text{HP (kJ)} = (16.18 \times \text{O}_2) + (5.02 \times \text{CO}_2) - (2.17 \times \text{CH}_4) - (5.99 \times \text{N}_{\text{urine}}) \]

where gases were expressed in liters per day and \text{N}_{\text{urine}} in grams per day. The body tissue energy (TE\text{body}) was calculated as MEI - HP - milk energy (E\text{milk}).
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The energy associated with the oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF) was calculated by the method of Brouwer (1958) and Chwalibog et al. (1997) for ruminants. The CO$_2$ production from oxidation (CO$_{2x}$) was calculated as CO$_2$ - (CO$_2$/CH$_4$ x CH$_4$), according to Fahey and Berger (1988). The calculations were carried as follows:

$$\text{OXP} = 6.25 \times N_{\text{urine}} \times 18.42 \text{ (kJ/g)},$$

$$\text{OXCHO} = [-2.968 \times O_2] + [4.174 \times \text{CO}_{2x}] - (2.446 \times N_{\text{urine}}] \times 17.58 \text{ (kJ/g)},$$

$$\text{OXF} = [(1.719 \times O_2) - (1.719 \times \text{CO}_{2x}) - (1.963 \times N_{\text{urine}}] \times 39.76 \text{ (kJ/g)}.$$

Then, the HP from oxidation was:

$$\text{HP}_x \text{ (kJ)} = (16.18 \times O_2) + (5.02 \times \text{CO}_{2x}) - (5.99 \times N_{\text{urine}})$$

Again, gases were expressed in liters per day and $N_{\text{urine}}$ in grams per day. Heat of fermentation (HPf) was estimated subtracting HP from HP$_x$. The non-protein respiratory quotient from oxidation of nutrients (RQ$_{npx}$) was determined as:

$$\text{RQ}_{npx} = \left[\text{CO}_{2x} - (N_{\text{urine}} \times 6.25 \times 0.774)\right] / \left[O_2 - (N_{\text{urine}} \times 6.25 \times 0.957)\right].$$

The efficiency of use of ME for lactation was calculated according to AFRC (1993). Energy lost from the body, indicating mobilization of body fat reserves in support of milk secretion, was assumed to be used for milk synthesis with an efficiency of 0.84 and the concomitant energy storage during lactation was taken to be 0.95 times the milk secretion efficiency. Consequently, the corrected milk energy was estimated as $E_{\text{milk}} + (0.84 \times \text{negative energy retention}) + (1.05 \times \text{positive energy retention})$. The efficiency of use of ME for milk production ($k_l$) was calculated as corrected milk energy/(ME - ME$_m$), with ME$_m$ being the metabolizable energy for maintenance, which was obtained from AFRC (1993) for goats (481 kJ/kg of BW$^{0.75}$).

In the C and N balance, we followed the equation and values proposed by McLean and Tobin (1987). The C balance includes the measurement of carbon in feed and that voided in feces, urine, CO$_2$, CH$_4$ and milk, while the N balance is based on the measurements on nitrogen in feed, feces, urine and milk. The C balance gave the total amount of C retained in the body and the amount of C
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retained in fat was calculated by subtracting the amount of C retained in protein determined by the N balance. Assuming an energy equivalent of 39.76 kJ/g and a content of 0.767 C for fat, and 23.86 kJ/g and 0.16 N and 0.52 C for protein, the energy retained (kJ) in protein (TE_{protein}) and fat (TE_{fat}) was calculated, respectively, as TE_{protein} = N balance (g) × 6.25 × 23.86, and TE_{fat} = (C balance (g) - N balance (g) × 6.25 × 0.52) × 1.304 × 39.76.

3.3.5. Statistical analyses

The effects of starch on intake, digestibility, metabolic energy as well as C and N balance were analyzed using the mixed model (proc MIXED) from SAS software (2001). The model for the dependent variables included the fixed effect of diet and period with goat as random effect. The following statistical model was used; Y = μ + D + T + goat + DxT + ε, where Y is the dependent variable, μ is the overall mean, D and T are the fixed effects of diet and period of time, DxT their interaction, goat is the random effect of goat and ε is the random error. Least square means are reported throughout and differences were considered significant at P < 0.05.

3.4. Results and discussion

3.4.1. Feed intake, digestibility and rumen fermentation

Dry matter intake (DMI) and total tract apparent digestibility of nutrients by Murciano-Granadina dairy goats are shown in Table 3.2. Not significant effect on DMI was observed for the diet, period of time and their interaction during the whole experiment (2.03 kg/d, on average). The diet was significant (P < 0.05) for DM, OM, CP, EE, ADF, starch and GE. The DM and GE total apparent tract digestibility were greater (P < 0.05) for BRL diet (71.6% and 73.4% respectively, both DM basis) and OP diet (73.5% and 73.4%, DM basis, respectively) than SH diet with more NDF content (64% and 67.2%, DM basis, respectively). The lower content of starch, higher ADF content and greater fat added in SH diet than BRL diet appeared to be the main factor responsible for the lower DM, OM and CP apparent digestibility found in SH diet. According to NRC (2001), soybean hulls
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feed is a by-product feed that is highly digestible but is low in non fibrous carbohydrates. However in our study NDF digestibility was lower, probably associated to the high fat content added to the diet. Difference ($P < 0.05$) was observed for the CP digestibility, being significant lower for OP diet (56.4%, DM basis) than BRL diet (65.6%, DM basis), and SH diet (59.3%, DM basis) did not differ from the other two diets. Accordingly, diet OP has lower CP content and lower CP digestibility than others, with a decrease in EE digestibility of 38 points regards to BRL and SH diets. Decreased digestibility in CP and EE in diet OP affected rumen fermentation as we observed in Table 3.3.

Rumen fermentation parameters obtained are displayed in Table 3.3. It was not possible to assess the effect of period and interaction with diet because rumen liquid extraction was done only during the second period of the trial. Lower pH values were found in OP and SH than BRL (7.1 vs. 7.3, respectively) and NH$_3$-N were greater in OP and SH than BRL (45.32 mg/dL for OP and SH on average vs. 15.08 mg/dL for BRL diet). An increase ($P < 0.05$) in acetic acid in the rumen was observed when NDF amount of diets increased (62.22 and 61.48 vs. 56.98 mol/100 mol for diet BRL, SH and OP, respectively). Butyric acid was higher ($P < 0.05$) in OP diet compared to other diets and the highest numerical value of NH$_3$-N was found in OP diet (52.3 mg/dL). The N-caproic acid and heptanoic acid showed significant ($P < 0.05$) differences between SH diet and OP or BRL diet. Therefore, in our study diets has the same source of protein (soybean meal) and different type of carbohydrate (starch, digestible and indigestible fiber), so the greater NH$_3$-N found in fibrous diets (OP and SH) seems indicative of their inefficient use for ruminal proteosynthesis (Casper et al., 1999). Besides, OP diet has lower level of CP than BRL and SH (12% vs. 14%, on average).

3.4.2. Energy balance

The average value obtained for the calibration factor, by releasing a known volume of N$_2$, CO$_2$ and CH$_4$ into the respirometry system, was 1.0070 ± 0.00119, 0.9979 ± 0.00823 and 0.955 ± 0.0061 for O$_2$, CO$_2$ and CH$_4$, respectively.
EXPERIMENT III: Replacement of barley grain with fibrous by-products

Daily energy balance obtained with the three diets is shown in Table 3.4. No significant differences were observed for GEI (2174.1 kJ/kg of BW\(^{0.75}\), on average) and significantly higher (\(P < 0.05\)) energy losses in feces (\(E_{\text{feces}}\)) were found for SH diet than BRL or OP diet. The OP and SH diets presented similar energy losses in urine (62.9 kJ/kg of BW\(^{0.75}\), on average) and were higher and significant (\(P < 0.001\)) than BRL (40.4 kJ/kg of BW\(^{0.75}\)), indicating that increasing the level of starch in diet reduces the energy losses in urine. The CH\(_4\) energy losses were not different among treatments. No differences were observed in MEI with averages values of 1352.4 kJ/kg of BW\(^{0.75}\). A significant effect of period of time (\(P < 0.05\)) was observed in HP, with greater values for the second one (848.6 kJ/kg of BW\(^{0.75}\) vs. 795.2 kJ/kg of BW\(^{0.75}\), respectively). No statistically significant differences were observed in \(E_{\text{milk}}\). The \(TE_{\text{body}}\) was significantly affected (\(P < 0.05\)) for the diet and period of time. Positive energy balance (80.5 kJ/kg of BW\(^{0.75}\)) was found for the starchy diet (BRL), around zero (1.3 kJ/kg of BW\(^{0.75}\)) in diet greater in NDF (SH) and negative energy balance (-9.2 kJ/kg of BW\(^{0.75}\)) in diet rich in digestible fiber (OP). Analyzing the period of time, almost zero energy balance (-5.1 kJ/kg of BW\(^{0.75}\)) was found in the first period and positive balance (74.1 kJ/kg of BW\(^{0.75}\)) in the second period (\(P < 0.05\)).

The efficiency of use of ME for milk production (\(k_i\)), according to AFRC (1993), was calculated as \(E_{\text{milk}}\) output adjusted to zero energy balance divided by ME-MEm, and MEm was obtained from the estimation according to AFRC (1993) for qm of 0.62 (481 kJ/kg of BW\(^{0.75}\)). No significant differences were observed between diets for \(k_i\) (0.61 on average) and the value was analogous to obtained by others authors. Aguilera et al. (1990) with lactating Granadina goats obtained a value of 0.67 and Tovar-Luna et al. (2010) with Alpine goats during mid lactation and 60% of concentrate found a value of 0.63. Bava et al. (2001) found a value for mixed diet in mid lactation of 0.67 for Saanen goats and López and Fernández (2013), with mixed diets and goats at mid lactation and positive energy balance, found values around 0.63.
3.4.3. Oxidation of nutrients

The CO₂ production is derived from nutrient oxidation and rumen fermentation. The separation between these two components is necessary to calculate the substrate oxidation in ruminants and determine the proportion of substrate oxidation supporting the total HP associated with oxidative processes. The proportional contribution to HPx due to oxidation of nutrients is shown in Table 3.5. No effect of interaction between diet and period was observed. Diet had no significant effect on HPx (789 kJ/kg of BW^{0.75}, on average). The significant (P < 0.05) effect of period of time was higher during a second period than first in OXP. A significant effect of diet (P < 0.05) was observed in HPf and OXF being higher for SH than the other two diets. The diets more fibrous (higher NDF in SH diet) were accompanied by greater HPf than starch diet (BRL) or digestible fiber (OP). The OXCHO was higher in BRL diet than others, possibly associated to the greater amount of starch in diet BRL than OP and SH.

Therefore, the heat from OXP contributed about 7.2% of HPx on average for three treatments. The BRL diet oxidized 59.2% of nutrients as OXCHO, and only 33.7% as OXF. However, the nutrients oxidation as OXF increased significantly (P < 0.05) to 76.6% in SH, and nutrients oxidation as OXCHO decreased significantly (P < 0.05) to 14.6%. The higher amount of NFC in OP diet promotes the higher OXCHO compared to SH diet. Nevertheless, OP diet presented intermediate values compared to the other two diets; the OXCHO and OXF was 45% and 49.2%, respectively. Few studies relating to oxidation of nutrients are available for ruminants and especially for small ruminants. In the study of López and Fernández (2013) in goats during mid lactation, total mixed diets bases in corn grain was replaced with a blend of fibrous by-product as soy hulls and corn gluten feed. In that trial no differences was observed between diets for OXCHO and OXF (55% and 29% on average, respectively). Hence, similar OXCHO was obtained than in our study but lower OXF than our trial. So, we expect similar OXF in our SH diet than the blend of fibrous by-product form López and Fernández (2013). These last authors added almost 4% of fat to the fibrous diet and it could be affect fiber fermentation (Palmquist and Jenkins, 1980). Chwalibog et al. (1997) in calves with
positive retained energy as fat pointed out that part of OXF should originate from ingested carbohydrate, mainly fiber. Significant difference \( P < 0.05 \) was observed for RQnpx, being significant lower for SH (0.76) than OP (0.84), and both lower than BRL (0.89). Chwalibog et al. (1997) reported that RQnpx lower than 1 indicates predominance of OXF vs. OXCHO, as we found in our study with diets OP and SH.

3.4.4. Carbon and nitrogen balance

The daily C and N balance and the calculated tissue recovered as protein and fat are displayed in Table 3.6. No effect of interaction between diet and period was observed with exception of C in milk. Significant differences were found for the excretion of C in feces in SH diet (19.8 g/kg of BW\(^{0.75} \)) than others (14.35 g/kg of BW\(^{0.75} \) on average). For C in CO\(_2\) expired also there was statistically different for both periods of time: 22.5 g/kg of BW\(^{0.75} \) in the second period of the trial and 20.8 g/kg of BW\(^{0.75} \) during the first. The C secreted into the milk was not significantly affected by treatment. The C retained was significantly affected \( (P < 0.05) \) for the effect of diet and period of time.

Goats of three groups ingested similar amounts of N (2.63 g/kg of BW\(^{0.75} \), on average) and no differences were found in excreted N (1.03 g/kg of BW\(^{0.75} \), on average). The N losses in urine were greater for SH treatment (0.6 g/kg of BW\(^{0.75} \)) when compared to BRL and OP (0.45 g/kg of BW\(^{0.75} \), on average). The N secreted into the milk was not affected by treatment (0.77 g/kg of BW\(^{0.75} \), on average). The N balance was different \( (P < 0.05) \) between OP and the other two diets. Although some authors (Kebreab et al., 2010) indicate reduction in urinary N output when ME intake increase, in our study we did not find differences.

The values of C and N retained in the body were converted to tissue energy recovered as protein or fat, and differences \( (P < 0.05) \) were found. The TE\(_{\text{protein}}\) was 64, 28.3 and 50 kJ/kg of BW\(^{0.75} \) for BRL, OP and SH, respectively. Regarding TE\(_{\text{fat}}\), the OP and SH diets showed greater \( (P < 0.05) \) fat mobilization (42.82 kJ/kg of BW\(^{0.75} \), on average) than BRL (19.2 kJ/kg of BW\(^{0.75} \)).
3.4.5. Milk production and fatty acids

Table 3.7 reports milk yield and chemical composition of the goats during the experiment. Significant ($P < 0.05$) and higher milk yield were found for the first period (2379.2 vs. 1931.2 g/d for first and second period, respectively). Milk dry matter and protein content were statistically different ($P < 0.05$) between the two periods with greater values for the second period than for the first one (16 vs. 14.5% for dry matter and 4.2 and 3.3% for protein content). No effect of interaction between diet and period was observed. Diet had no effect on milk yield (average milk yield was 2155.2 g/d), dry matter, fat, protein and lactose (15.25%, 6.0%, 3.75% and 4.6% on average, respectively). Although increased milk fat content is common when dietary fiber concentrations rise at the expense of starch, no differences was found in our study.

Effect of diet on the fatty acid profile of milk fat is shown in Table 3.8. No effect of diet was observed in fatty acids with 4 to 15 carbon atoms. However, there were significant differences between diets in most of the other fatty acids. The fatty acids with 16 or fewer carbon atoms derive from de novo synthesis, whereas those with 18 or more carbons atoms come from the diet or from lipid mobilization (Chilliard et al., 2003). Significant differences ($P < 0.05$) were observed for palmitic acid and cis-10-heptadecenoic acid, being significant higher for OP diet than BRL diet, and SH diet did not differ from the other two diets. Pentadecanoic acid and heptadecanoic acid are potential biomarkers of rumen function since they are found in rumen bacterial lipids and might be partially synthesized endogenously from rumen substrates in the mammary gland (Vlaeminck et al., 2006, Fievez et al., 2012 and Vlaeminck et al., 2015). The differences ($P < 0.05$) found between treatments (lower content of pentanoic acid in the milk of OP and SH goats than BRL) suggest a negative impact of these diets on rumen bacterial metabolism and the fermentative activity. Our ammonia-N results found in rumen liquid are in accordance with the differences observed in these fatty acids. Besides, greater amount ($P < 0.05$) of heptadecanoic acid in milk fat from OP and SH diets probably reflect a larger contribution of mobilized fat (Fievez et al., 2012), as we observed in Table 3.6; -
EXPERIMENT III: Replacement of barley grain with fibrous by-products

42.82 kJ TE_{tot}/kg of BW^{0.75} in OP and SH diet on average against 19.2 kJ TE_{tot}/kg of BW^{0.75} in BRL. Vlaeminck et al. (2006) suggest that the amount of starch in the diet is an important factor determining high milk eladic acid content (greater in BRL than others). Milk fat of goats fed BRL diet had lower percentages of vaccenic acid and higher of linoleic acid and cis-11-eicosenoic acid than the other two diets. Higher vaccenic acids in OP and SH diets seems to be positively correlated with negative energy balances in goats. Jorjong et al. (2015) found positive correlation between negative energy balance and oleic acid in dairy cow. Goats fed OP diet had greater percentages of linolenic acid and CLA 10t12c in their milk fat than goats fed BRL or SH diet. Significant differences were observed for CLA 9c11t + 9t11c and arachidonic acid, being significant higher for BRL diet than SH diet, and OP diet did not differ from the other two diets. Milk from goats fed BRL diet is the richest in medium-chain fatty acids and polyunsaturated fatty acids. Milk from goats fed SH diet is the richest in monounsaturated fatty acids and goats fed OP diet have milk with more saturated fatty acids. Atherogenicity index was calculated as indicate by Ulbricht and Southgate (1991). The milk of goats fed SH diet has the lower atherogenicity index than others (4.42 vs. 5.4 on average, respectively), but differences were not significant.

3.4.6. Methane emission

Table 3.9 shows enteric CH\textsubscript{4} emissions from goats. The average CH\textsubscript{4} emission from the goat’s digestive tracts (enteric fermentation) was similar between diets and averaged at 40.7 g/goat per day. Methane conversion ratio, also called Ym factor, represents energy loss as CH\textsubscript{4} per unit of GE intake. No differences were observed and average Ym value was 6.4. According to Johnson and Johnson (1995) and Knapp et al. (2014), fermentation of fibrous carbohydrates produces more CH\textsubscript{4} than fermentation of soluble sugars, which in turn produce more CH\textsubscript{4} than fermentation of starch. The similar CH\textsubscript{4} production that we found in this work can be explained by the fact that greater NH\textsubscript{3}-N found in OP diet probably cause asynchrony in rumen fermentation. Regards to SH diet, the higher fat added (almost 2% vs. 0.3% for SH and others, respectively) probably affected fiber degradation. Significant (P < 0.05) higher g CH\textsubscript{4}/kg milk was observed in the
second period of the trial. No effect of interaction between diet and period was observed. No effects of CH₄ emission were found when it is related to DM or OM.

3.4.7. Metabolites in milk, urine and plasma

Table 3.10 shows metabolites from goats. It was not possible to assess the effect of period and interaction with diet because blood samples were obtained only during the second period of the trial. The lower NH₃-N found in diet BRL was followed by greater values ($P < 0.05$) of urea in urine, plasma urea and milk urea than the values found in OP diet, while SH diet was similar to BRL. The review of Spek et al. (2013) reported the effect of the type of carbohydrate on rumen NH₃ utilization and observed that starch and glucose rich diets resulted in lower concentration of rumen ammonia, plasma urea nitrogen and milk urea nitrogen. But in our study, plasma urea nitrogen and milk urea nitrogen was similar between BRL and SH diets. Although the type of carbohydrate affect rumen microbial protein synthesis, in our study fat mobilization to meet energy requirements was found. The TEfat shown in Table 3.6 was -40.60 and -45 kJ/ kg of BW⁰.⁷⁵ for OP and SH, respectively. High rates of fat mobilization lead to markedly increase ($P < 0.05$) in plasma concentrations of NEFA, BHBA and accumulation of triglycerides. These differences were found between OP and others, but no between OP and SH. Besides plasma glucose was greater in BRL than OP. During negative energy balance blood glucose concentration is low and body fat is mobilized and transported as NEFA to several organs, particularly to the liver in which these FA are oxidized to produce energy. Excessive amounts of NEFA released during body fat mobilization are transferred to the milk. The major NEFA released are palmitic acid, stearic acid and oleic acid, and their elevated concentration in milk fat of those FA were identified as valuable early warning biomarkers for negative energy balance (Jorjong et al., 2015). Greater ($P < 0.05$) concentration of palmitic acid, heptadecanoic acid and vaccenic acid were found in OP and SH than BRL, and the highest value of oleic acid was found in SH diet.

Therefore, we observed that BRL diet suggest that glucogenic nutrients stimulate body fat deposition and the partitioning of ME into body tissues and
milk (most of the HPx derived from OXCHO: 59.2%). The other two diets were lipogenic diets, and lipogenic nutrients originate either from fiber, or dietary fat or from body reserves (Van Knegsel et al., 2007). Fat mobilization was found in OP and SH, although greater OXF (76.6%) was found in SH than OP (49.2%). Therefore, BRL behave as glucogenic diet, SH as lipogenic diet and OP intermediate, but due to possible asynchrony between protein degradation and type of carbohydrate the NH₃-N increased in OP and SH and fat mobilization enlarged to meet energy demand and milk performance.

3.5. Conclusions

This paper provides data on energy partitioning, substrate oxidation, carbon and nitrogen balances, methane emissions and milk performance in Murciano-Granadina goats during mid lactation fed mixed diets. The replacement of 59% of barley on the diet by dry orange pulp or soybean hulls did not affect milk yield (2,155.2 g/d) and no effect was found for chemical composition. The higher starch diet resulted in positive energy balance and higher fibrous diets shown fat mobilization. The differences found between treatments (lower contents of pentadecanoic acid and higher in heptadecanoic acid in the goat milk fed fibrous by-product) suggest a negative impact of these diets on rumen bacterial fermentative activity and fat mobilization. Replacement of cereal grain with fibrous by-products did not increased methane emissions. Therefore, lactating goats could utilize dry orange pulp and soybean hulls diets without detrimental effect on milk performance, although attention should be paid to fat added to the diet, and the synchrony between diet fiber and the source of protein used.

3.6. Acknowledgements

This study was supported by the Spanish National Institute for Agronomic Research (RTA2011-00107-C02) and the European Regional Development Fund (ERDF).
EXPERIMENT III: Replacement of barley grain with fibrous by-products

3.7. References


EXPERIMENT III: Replacement of barley grain with fibrous by-products


EXPERIMENT III: Replacement of barley grain with fibrous by-products


EXPERIMENT III: Replacement of barley grain with fibrous by-products

## Table 3.1. Ingredients and chemical composition of the diets.

<table>
<thead>
<tr>
<th>Ingredients, g/kg DM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Diet&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BRL</th>
<th>OP</th>
<th>SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa hay</td>
<td></td>
<td>330.0</td>
<td>330.0</td>
<td>330.0</td>
</tr>
<tr>
<td>Barley</td>
<td></td>
<td>594.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Orange pulp</td>
<td></td>
<td>-</td>
<td>593.7</td>
<td>-</td>
</tr>
<tr>
<td>Soy hulls</td>
<td></td>
<td>-</td>
<td>-</td>
<td>595.3</td>
</tr>
<tr>
<td>Soy meal 44% crude protein</td>
<td></td>
<td>46.8</td>
<td>59.3</td>
<td>39.6</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td></td>
<td>11.8</td>
<td>7.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td></td>
<td>8.7</td>
<td>2.7</td>
<td>7.5</td>
</tr>
<tr>
<td>Bypass fat&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>4.2</td>
<td>2.5</td>
<td>19.1</td>
</tr>
<tr>
<td>Premix&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>4.2</td>
<td>4.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>

### Chemical composition, % of DM

<table>
<thead>
<tr>
<th>Dry matter</th>
<th>BRL</th>
<th>OP</th>
<th>SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter</td>
<td>92.8</td>
<td>91.0</td>
<td>91.4</td>
</tr>
<tr>
<td>Crude protein</td>
<td>13.7</td>
<td>11.9</td>
<td>14.0</td>
</tr>
<tr>
<td>Ether extract</td>
<td>2.2</td>
<td>1.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>41.5</td>
<td>31.4</td>
<td>54.6</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>15.8</td>
<td>20.8</td>
<td>38.2</td>
</tr>
<tr>
<td>NFC&lt;sup&gt;e&lt;/sup&gt;</td>
<td>35.5</td>
<td>46.6</td>
<td>21.0</td>
</tr>
<tr>
<td>Starch</td>
<td>32.7</td>
<td>5.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Gross energy, MJ/kg DM&lt;sup&gt;f&lt;/sup&gt;</td>
<td>17.9</td>
<td>16.9</td>
<td>18.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>BRL = barley; OP = orange pulp; SH = soybean hulls.

<sup>b</sup>DM = dry matter

<sup>c</sup>Bypass fat of palm fatty acid distillate. Provided by Norel Animal Nutrition, Norel S.A., Spain.

<sup>d</sup>Provided by NACOOP S.A. España. Premix composition (ppm or UI per kilogram of premix): Se, 40; I, 250; Co, 80; Cu, 3000; Fe, 6000; Zn, 23400; Mn, 29000; S, 60000; Mg, 60000; vitamin A, 2000000 UI; vitamin D3, 400000; vitamin E, 2000 ppm; nicotinic acid, 10000; choline, 20300.

<sup>e</sup>NFC = non fibrous carbohydrate content: 100 - (NDF + ash + CP + EE).
### Table 3.2. Body weight, intake, and apparent digestibility coefficients of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items</th>
<th>Diet(^1)</th>
<th>Period(^2)</th>
<th>SEM(^3)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRL</td>
<td>OP</td>
<td>SH</td>
<td>1</td>
</tr>
<tr>
<td>BW, kg</td>
<td>42.4</td>
<td>42.3</td>
<td>41.6</td>
<td>42.2</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>2.0</td>
<td>2.0</td>
<td>2.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Digestibility, % of DM**

<table>
<thead>
<tr>
<th>Items</th>
<th>Diet(^1)</th>
<th>Period(^2)</th>
<th>SEM(^3)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>71.6(^a)</td>
<td>73.5(^a)</td>
<td>64.0(^b)</td>
<td>70.3</td>
</tr>
<tr>
<td>OM</td>
<td>74.5(^b)</td>
<td>77.5(^c)</td>
<td>66.6(^c)</td>
<td>73.4</td>
</tr>
<tr>
<td>CP</td>
<td>65.6(^a)</td>
<td>56.4(^b)</td>
<td>59.3(^ab)</td>
<td>60.9</td>
</tr>
<tr>
<td>EE</td>
<td>74.2(^a)</td>
<td>34.0(^a)</td>
<td>71.1(^a)</td>
<td>60.0</td>
</tr>
<tr>
<td>NDF</td>
<td>64.0</td>
<td>61.4</td>
<td>60.8</td>
<td>63.6</td>
</tr>
<tr>
<td>ADF</td>
<td>47.9(^c)</td>
<td>65.6(^a)</td>
<td>58.7(^a)</td>
<td>55.6</td>
</tr>
<tr>
<td>Starch</td>
<td>99.4(^a)</td>
<td>98.2(^c)</td>
<td>91.9(^c)</td>
<td>97.0</td>
</tr>
<tr>
<td>GE</td>
<td>73.4(^a)</td>
<td>73.4(^a)</td>
<td>67.2(^a)</td>
<td>70.3</td>
</tr>
</tbody>
</table>

\(^{a,c}\)Means within a row with different superscripts differ (P < 0.05).
\(^1\)BRL = barley; OP = orange pulp; SH = soybean hulls.
\(^2\)1 = first period; 2 = second period.
\(^3\)SEM = standard error of the mean.
\(^4\)BW = body weight; BW\(^0.75\) = metabolic body weight; DMI = dry matter intake; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; GE = gross energy.
Table 3.3. pH, ammonia-N (NH3-N), and VFA of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items</th>
<th>Diet</th>
<th>SEM2</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRL</td>
<td>OP</td>
<td>SH</td>
</tr>
<tr>
<td>pH</td>
<td>7.30a</td>
<td>7.10b</td>
<td>7.10b</td>
</tr>
<tr>
<td>NH3-N, mg/dL</td>
<td>15.08b</td>
<td>52.30a</td>
<td>38.33a</td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>14.16c</td>
<td>25.97a</td>
<td>23.94b</td>
</tr>
<tr>
<td>Individual VFA, mol/100 mol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>62.22a</td>
<td>56.98b</td>
<td>61.48a</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>13.42</td>
<td>14.98</td>
<td>15.90</td>
</tr>
<tr>
<td>Isobutric acid</td>
<td>3.86</td>
<td>2.87</td>
<td>3.19</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>14.39b</td>
<td>19.54a</td>
<td>13.09b</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>4.34</td>
<td>3.81</td>
<td>3.74</td>
</tr>
<tr>
<td>N-Valeric acid</td>
<td>1.78</td>
<td>1.70</td>
<td>1.74</td>
</tr>
<tr>
<td>N-Caproic acid</td>
<td>0.00b</td>
<td>0.12a</td>
<td>0.62a</td>
</tr>
<tr>
<td>Heptanoic acid</td>
<td>0.00b</td>
<td>0.00b</td>
<td>0.23a</td>
</tr>
</tbody>
</table>

a-c Means within a row with different superscripts differ (P < 0.05).

1BRL = barley; OP = orange pulp; SH = soybean hulls.
2SEM = standard error of the mean.
3NH3-N = ammonia nitrogen; VFA = volatile fatty acids; N = nitrogen.
### Table 3.4. Daily energy partitioning (kJ/kg of BW<sup>0.75</sup>) of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Diet&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Period&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-Value</th>
<th>Diet</th>
<th>Period</th>
<th>Diet x Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRL</td>
<td>OP</td>
<td>SH</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GEI</td>
<td>2170.2</td>
<td>2055.8</td>
<td>2296.2</td>
<td>2142.5</td>
<td>2205.6</td>
<td>42.66</td>
<td>0.113</td>
</tr>
<tr>
<td>E&lt;sub&gt;feces&lt;/sub&gt;</td>
<td>582.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>545.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>758.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>651.6</td>
<td>605.8</td>
<td>26.06</td>
<td>0.001</td>
</tr>
<tr>
<td>E&lt;sub&gt;urine&lt;/sub&gt;</td>
<td>40.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.3</td>
<td>60.4</td>
<td>5.24</td>
<td>0.041</td>
</tr>
<tr>
<td>E&lt;sub&gt;methylene&lt;/sub&gt;</td>
<td>135.9</td>
<td>141.5</td>
<td>135.4</td>
<td>129.7</td>
<td>145.5</td>
<td>4.60</td>
<td>0.852</td>
</tr>
<tr>
<td>MEI</td>
<td>1411.7</td>
<td>1312.5</td>
<td>1333.0</td>
<td>1310.9</td>
<td>1393.9</td>
<td>29.51</td>
<td>0.301</td>
</tr>
<tr>
<td>HP</td>
<td>817.6</td>
<td>847.1</td>
<td>803.8</td>
<td>795.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>848.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.06</td>
<td>0.471</td>
</tr>
<tr>
<td>E&lt;sub&gt;milk&lt;/sub&gt;</td>
<td>513.5</td>
<td>474.6</td>
<td>527.8</td>
<td>572.0</td>
<td>492.3</td>
<td>14.65</td>
<td>0.473</td>
</tr>
<tr>
<td>T&lt;sub&gt;Ebody&lt;/sub&gt;</td>
<td>80.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-9.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-5.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.06</td>
<td>0.017</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means within a row with different superscripts differ (P < 0.05).

<sup>1</sup>BRL = barley; OP = orange pulp; SH = soybean hulls.

<sup>2</sup>1 = first period; 2 = second period.

<sup>3</sup>SEM = standard error of the mean.

<sup>4</sup>GEI = gross energy intake; E<sub>feces</sub> = energy losses in feces; E<sub>urine</sub> = energy losses in urine; E<sub>methylene</sub> = energy losses in methane; MEI = metabolizable energy intake; HP = heat production; E<sub>milk</sub> = milk energy; T<sub>Ebody</sub> = recovered energy in tissue (T<sub>Ebody</sub> = MEI − HP − E<sub>milk</sub>).
Table 3.5. Heat production (kJ/kg of BW\(^{0.75}\)) from oxidation and fermentation; daily oxidation (kJ/kg of BW\(^{0.75}\)) of protein, carbohydrate, and fat; and their contribution to the heat production from oxidation substrates (%) of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items(^4)</th>
<th>Diet(^1)</th>
<th>Period(^2)</th>
<th>SEM(^3)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRL</td>
<td>OP</td>
<td>SH</td>
<td>1</td>
</tr>
<tr>
<td>HPx</td>
<td>795.7</td>
<td>811.7</td>
<td>759.7</td>
<td>765.6</td>
</tr>
<tr>
<td>HPf</td>
<td>21.9(^c)</td>
<td>35.4(^b)</td>
<td>44.1(^a)</td>
<td>29.7</td>
</tr>
<tr>
<td>OXP</td>
<td>56.0</td>
<td>47.5</td>
<td>66.4</td>
<td>49.1(^b)</td>
</tr>
<tr>
<td>OXCHO</td>
<td>471.9(^a)</td>
<td>363.0(^b)</td>
<td>115.6(^c)</td>
<td>329.0</td>
</tr>
<tr>
<td>OXF</td>
<td>267.7(^c)</td>
<td>401.1(^b)</td>
<td>577.5(^a)</td>
<td>387.3</td>
</tr>
<tr>
<td>OXP/HPx</td>
<td>7.1</td>
<td>5.8</td>
<td>8.8</td>
<td>6.4</td>
</tr>
<tr>
<td>OXCHO/HPx</td>
<td>59.2(^a)</td>
<td>45.0(^b)</td>
<td>14.6(^c)</td>
<td>42.1</td>
</tr>
<tr>
<td>OXF/HPx</td>
<td>33.7(^c)</td>
<td>49.2(^b)</td>
<td>76.6(^a)</td>
<td>51.5</td>
</tr>
<tr>
<td>RQnpx</td>
<td>0.89(^a)</td>
<td>0.84(^b)</td>
<td>0.76(^c)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

\(^a-c\) Means within a row with different superscripts differ (P < 0.05).

\(^1\)BRL = barley; OP = orange pulp; SH = soybean hulls.

\(^2\)1 = first period; 2 = second period.

\(^3\)SEM = standard error of the mean.

\(^4\)HPx = heat production from oxidation of nutrients; HPf = heat production of fermentation [HPf = HP - HPx (Brouwer, 1958)]; OXP = heat production associated with the oxidation of protein; OXCHO = heat production associated with the oxidation of carbohydrates; OXF = heat production associated with the oxidation of fat; RQnpx = non protein respiratory quotient (unitless) from oxidation of nutrients \([\frac{\text{CO}_2 \times [\text{N}_{\text{urine}} \times 6.25 \times 0.774]}{\text{O}_2 \times [\text{N}_{\text{urine}} \times 6.25 \times 0.957]}\] \), where CO\(_2\) = CO\(_2\) production from oxidation and N\(_{\text{urine}}\) = N in urine.
Table 3.6. Carbon and nitrogen balance (g/kg of BW\(^{0.75}\)) of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items(^4)</th>
<th>Diet(^1)</th>
<th>Period(^2)</th>
<th>SEM(^3)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRL</td>
<td>OP</td>
<td>SH</td>
<td>1</td>
</tr>
<tr>
<td>C(_{\text{intake}})</td>
<td>52.4</td>
<td>50.1</td>
<td>56.1</td>
<td>52.0</td>
</tr>
<tr>
<td>C(_{\text{feces}})</td>
<td>15.0(^b)</td>
<td>13.7(^b)</td>
<td>19.8(^a)</td>
<td>15.7</td>
</tr>
<tr>
<td>C(_{\text{urine}})</td>
<td>1.3(^b)</td>
<td>1.6(^b)</td>
<td>1.8(^b)</td>
<td>1.5</td>
</tr>
<tr>
<td>C(_{\text{CO2}})</td>
<td>21.5</td>
<td>22.6</td>
<td>21.1</td>
<td>20.8(^b)</td>
</tr>
<tr>
<td>C(_{\text{CH4}})</td>
<td>1.8</td>
<td>1.9</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>C(_{\text{milk}})</td>
<td>11.1</td>
<td>10.5</td>
<td>11.4</td>
<td>11.6</td>
</tr>
<tr>
<td>C(_{\text{retained body}})</td>
<td>1.8(^a)</td>
<td>0.2(^b)</td>
<td>-0.2(^c)</td>
<td>1.1(^a)</td>
</tr>
<tr>
<td>N(_{\text{intake}})</td>
<td>2.7</td>
<td>2.3</td>
<td>2.9</td>
<td>2.6</td>
</tr>
<tr>
<td>N(_{\text{feces}})</td>
<td>0.9</td>
<td>1.0</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>N(_{\text{urine}})</td>
<td>0.5(^b)</td>
<td>0.4(^b)</td>
<td>0.6(^a)</td>
<td>0.4(^b)</td>
</tr>
<tr>
<td>N(_{\text{milk}})</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>N(_{\text{retained body}})</td>
<td>0.4(^a)</td>
<td>0.2(^b)</td>
<td>0.3(^a)</td>
<td>0.4</td>
</tr>
<tr>
<td>TE(_{\text{protein}}, \text{kJ/kg of BW}(^{0.75}))</td>
<td>64.0(^a)</td>
<td>28.3(^c)</td>
<td>50.0(^b)</td>
<td>58.5</td>
</tr>
<tr>
<td>TE(_{\text{fat}}, \text{kJ/kg of BW}(^{0.75}))</td>
<td>19.2(^a)</td>
<td>-40.6(^c)</td>
<td>-45.0(^b)</td>
<td>-9.3(^b)</td>
</tr>
</tbody>
</table>

\(^a\)^ Means within a row with different superscripts differ (P < 0.05).

\(^1\)BRL = barley; OP = orange pulp; SH = soybean hulls.

\(^2\)1 = first period; 2 = second period.

\(^3\)SEM = standard error of the mean.

\(^4\)C\(_{\text{intake}}\) = C intake; C\(_{\text{feces}}\) = C losses in feces; C\(_{\text{urine}}\) = C losses in urine; C\(_{\text{CO2}}\) = C losses in CO\(_2\); C\(_{\text{CH4}}\) = C losses in methane; C\(_{\text{milk}}\) = recovered C in milk; C\(_{\text{retained body}}\) = recovered C in tissue; N\(_{\text{intake}}\) = N intake; N\(_{\text{feces}}\) = N losses in feces; N\(_{\text{urine}}\) = N losses in urine; N\(_{\text{milk}}\) = recovered N in milk; N\(_{\text{retained body}}\) = recovered N in tissue; TE\(_{\text{body}}\) = recovered energy in tissue (TE\(_{\text{body}}\) = MEI − HP − E\(_{\text{milk}}\)).
**Table 3.7.** Daily milk production and composition of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items</th>
<th>Diet&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Period&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRL</td>
<td>OP</td>
<td>SH</td>
<td>1</td>
</tr>
<tr>
<td>Milk yield, g/goat/day</td>
<td>2285.4</td>
<td>2013.8</td>
<td>2166.5</td>
<td>2379.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Composition, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM&lt;sup&gt;4&lt;/sup&gt;</td>
<td>14.7</td>
<td>15.7</td>
<td>15.4</td>
<td>14.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>5.4</td>
<td>6.3</td>
<td>6.4</td>
<td>5.7</td>
</tr>
<tr>
<td>Protein</td>
<td>4.0</td>
<td>3.7</td>
<td>3.7</td>
<td>3.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.6</td>
<td>4.7</td>
<td>4.5</td>
<td>4.6</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row with different superscripts differ (P < 0.05).

<sup>1</sup>BRL = barley; OP = orange pulp; SH = soybean hulls.

<sup>2</sup>1 = first period; 2 = second period.

<sup>3</sup>SEM = standard error of the mean.

<sup>4</sup>DM = dry matter.
### Table 3.8. Fatty acid composition (g/100 g of identified fatty acids) of milk fat for goats (n = 12) fed experimental diets.

<table>
<thead>
<tr>
<th>Items</th>
<th>Diet</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRL</td>
<td>OP</td>
<td>SH</td>
</tr>
<tr>
<td>Butyric Acid</td>
<td>0.30</td>
<td>0.32</td>
<td>0.31</td>
</tr>
<tr>
<td>Caproic Acid</td>
<td>0.95</td>
<td>0.97</td>
<td>0.92</td>
</tr>
<tr>
<td>Caprylic Acid</td>
<td>1.82</td>
<td>1.74</td>
<td>1.68</td>
</tr>
<tr>
<td>Capric Acid</td>
<td>10.03</td>
<td>9.60</td>
<td>8.81</td>
</tr>
<tr>
<td>Undecanoic Acid</td>
<td>0.37</td>
<td>0.32</td>
<td>0.29</td>
</tr>
<tr>
<td>Lauric Acid</td>
<td>6.89</td>
<td>5.99</td>
<td>5.46</td>
</tr>
<tr>
<td>Myristic Acid</td>
<td>13.38</td>
<td>13.13</td>
<td>11.94</td>
</tr>
<tr>
<td>Myristoleic Acid</td>
<td>0.28</td>
<td>0.30</td>
<td>0.27</td>
</tr>
<tr>
<td>Pentadecanoic Acid</td>
<td>0.22</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>40.61</td>
<td>44.53</td>
<td>42.63</td>
</tr>
<tr>
<td>Palmitoleic Acid</td>
<td>1.11</td>
<td>1.36</td>
<td>1.27</td>
</tr>
<tr>
<td>Heptadecanoic Acid</td>
<td>0.59</td>
<td>0.70</td>
<td>0.71</td>
</tr>
<tr>
<td>cis-10-Heptadecanoic Acid</td>
<td>0.28</td>
<td>0.36</td>
<td>0.33</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>4.69</td>
<td>3.77</td>
<td>4.96</td>
</tr>
<tr>
<td>Elaidic Acid</td>
<td>0.37</td>
<td>0.32</td>
<td>0.23</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>13.43</td>
<td>12.45</td>
<td>16.70</td>
</tr>
<tr>
<td>Vaccenic Acid</td>
<td>0.31</td>
<td>0.46</td>
<td>0.41</td>
</tr>
<tr>
<td>Linoleic Acid</td>
<td>3.18</td>
<td>2.25</td>
<td>1.95</td>
</tr>
<tr>
<td>Arachidic Acid</td>
<td>0.11</td>
<td>0.10</td>
<td>0.16</td>
</tr>
<tr>
<td>gamma-Linolenic Acid</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>cis-11-Eicosenoic Acid</td>
<td>0.07</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Linolenic Acid</td>
<td>0.35</td>
<td>0.52</td>
<td>0.34</td>
</tr>
<tr>
<td>CLA 9c11t + 9t11c</td>
<td>0.47</td>
<td>0.41</td>
<td>0.28</td>
</tr>
<tr>
<td>CLA 10t12c</td>
<td>0.01</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Arachidonic Acid</td>
<td>0.19</td>
<td>0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>Medium-chain fatty acids</td>
<td>20.04</td>
<td>18.62</td>
<td>17.16</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>15.84</td>
<td>15.30</td>
<td>19.25</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>4.22</td>
<td>3.38</td>
<td>2.72</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>79.94</td>
<td>81.32</td>
<td>78.02</td>
</tr>
<tr>
<td>AI</td>
<td>5.15</td>
<td>5.64</td>
<td>4.42</td>
</tr>
</tbody>
</table>

---

*Means within a row with different superscripts differ (P < 0.05).

1BRL = barley; OP = orange pulp; SH = soybean hulls.

2SEM = standard error of the mean.

3CLA = conjugated linoleic acid; AI = Atherogenicity index calculated as C12:0 + 4 × C14:0 + C16:0/unsaturated fatty acids (Ulbricht and Southgate, 1991).
Table 3.9. Methane emission of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items(^4)</th>
<th>Diet(^1)</th>
<th>Period(^2)</th>
<th>SEM(^3)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRL</td>
<td>OP</td>
<td>SH</td>
<td>1</td>
</tr>
<tr>
<td>CH(_4), g/d</td>
<td>40.5</td>
<td>42.3</td>
<td>39.4</td>
<td>38.8</td>
</tr>
<tr>
<td>Ym, %</td>
<td>6.3</td>
<td>6.9</td>
<td>5.9</td>
<td>6.1</td>
</tr>
<tr>
<td>CH(_4)/DMi, g/kg</td>
<td>20.1</td>
<td>21.1</td>
<td>19.0</td>
<td>19.3</td>
</tr>
<tr>
<td>CH(_4)/OMi, g/kg</td>
<td>21.7</td>
<td>23.1</td>
<td>20.7</td>
<td>20.9</td>
</tr>
<tr>
<td>CH(_4)/milk, g/kg</td>
<td>18.3</td>
<td>21.4</td>
<td>18.4</td>
<td>16.2</td>
</tr>
</tbody>
</table>

\(a,b\) Means within a row with different superscripts differ (\(P < 0.05\)).
\(^1\)BRL = barley; OP = orange pulp; SH = soybean hulls.
\(^2\)1 = first period; 2 = second period.
\(^3\)SEM = standard error of the mean.
\(^4\)Ym = methane energy/gross energy intake; DMi = dry matter intake; OMi = organic matter intake.
Table 3.10. Metabolites in milk, urine and plasma of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

<table>
<thead>
<tr>
<th>Items</th>
<th>Diet$^1$</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRL</td>
<td>OP</td>
<td>SH</td>
<td>SEM$^2$</td>
<td>P-Value</td>
</tr>
<tr>
<td><strong>Milk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea$_{\text{milk}}$, mg/L</td>
<td>419.6$^a$</td>
<td>294.5$^b$</td>
<td>393.8$^a$</td>
<td>28.10</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein, mg/L</td>
<td>147.5$^b$</td>
<td>205.0$^a$</td>
<td>103.3$^b$</td>
<td>15.34</td>
<td>0.014</td>
</tr>
<tr>
<td>Urea, mg/L</td>
<td>16012.5$^a$</td>
<td>10500.0$^b$</td>
<td>9916.7$^c$</td>
<td>111.91</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/L</td>
<td>510.0$^a$</td>
<td>390.0$^b$</td>
<td>416.7$^{ab}$</td>
<td>42.29</td>
<td>0.038</td>
</tr>
<tr>
<td>NEFA$^3$, mM/L</td>
<td>0.9$^b$</td>
<td>1.4$^a$</td>
<td>0.7$^b$</td>
<td>0.12</td>
<td>0.017</td>
</tr>
<tr>
<td>Beta-Hydroxybutyrate, mM/L</td>
<td>1.1$^b$</td>
<td>3.2$^a$</td>
<td>0.8$^b$</td>
<td>0.44</td>
<td>0.010</td>
</tr>
<tr>
<td>Ketone bodies, mM/L</td>
<td>1.2$^a$</td>
<td>3.3$^a$</td>
<td>0.9$^b$</td>
<td>0.46</td>
<td>0.010</td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>37.5$^b$</td>
<td>80.0$^a$</td>
<td>60.0$^{ab}$</td>
<td>13.55</td>
<td>0.045</td>
</tr>
<tr>
<td>Urea, mg/L</td>
<td>389.0$^a$</td>
<td>200.0$^{ab}$</td>
<td>330.0$^a$</td>
<td>22.24</td>
<td>0.042</td>
</tr>
</tbody>
</table>

$^a$-$c$ Means within a row with different superscripts differ ($P < 0.05$).
$^1$BRL = barley; OP = orange pulp; SH = soybean hulls.
$^2$SEM = standard error of the mean.
$^3$NEFA = non esterified fatty acids.
GENERAL DISCUSSION
The aims of this research were to investigate the influence of diet carbohydrates on methane production, also the effect on milk performance, digestibility, rumen parameters, energy partitioning, carbon and nitrogen balance or substrate oxidation.

It is often claimed that forage based diets generally results in considerably higher enteric CH₄ formation than mixed or concentrated based diets, as well high starch content in diet reduces the CH₄ production (Beauchemin et al., 2008). Reports in the literature also indicates that inclusion of fibrous by-products in the diet of ruminant will increase enteric CH₄ particularly when inclusion is above 35% to 40% of DMI.

In our first experiment, the 30% of corn grain was replaced with beet pulp, and the CH₄ production for this beet pulp diet was higher than for the corn diet, indicating that, really, increasing the level of starch in diet reduces the CH₄ production. The higher ammonia N observed in the corn diet is the responsible of less fermentative activity and lower CH₄ production, too.

In the second experiment, the average CH₄ emissions from the goat’s digestive tracts was similar between diets. The inclusion of fibrous by-products was lower than 35% of DMI and the highly fermentable fiber is very different from fiber forages that are usually associated to CH₄ production. Lack of synchronization of carbohydrates and protein in low starch diet and the higher level of fat added to this diet were the responsible of the absence of effect of low starch diet on CH₄ enteric emissions. High acetic acid productions are accompanied with high levels of available hydrogen and hence with high methane production. However, soluble sugars modify the process. Fiber by-products contain more soluble sugars than roughage. Greater proportion of butyric acid was observed in low starch diet compared with the high starch one, because fiber quality of by-products could be support fermentation processes to butyric acid.

In our last experiment, replacement of cereal grain (59%) with fibrous by-products did not increased methane emissions. The average CH₄ emission from enteric fermentation was similar between diets and no differences were
observed in Ym. However, the amount of $\text{CH}_4$ emitted in relation to the ingested and digested NDF was higher with the orange pulp diet compared with the barley or soybean hulls diet. As in the second experiment, fat was added to the food in order to increase the energy diet content due that cereal grain was replaced with fibrous by-products.

Therefore, the use of mixed diets in lactating Murciano-Granadina goats replacing cereal grain with fibrous by-product did not decrease milk performance and did not increase methane emission; the average of methane emission from the three experiments was 30 g/d and the methane energy loss was 5% of the gross energy intake on average. Methane emitted from ruminants represents a loss from 2% (grain diet) to 12% (forage diet) of the ruminant’s gross energy intake (Johnson and Johnson, 1995), and in our study the value obtained was intermediate. Wilkerson et al. (1995) found that $\text{CH}_4$ emission in cattle ranged from 6% to 10%. Patra and Lalhriatpuii (2016) suggested that $\text{CH}_4$ production is lower in goats than in cattle due to greater passage rate of feeds for goats than for cattle, and consequently low $\text{CH}_4$ production per unit of feed intake for goats.

As we discussed previously, many nutritional factors influence the ruminal fermentation, but in our studies we had to incorporate more variation factors. For instance, the indirect calorimeter used in the first experiment had a face mask and the other two a head hood. First experiment was run during late lactation and the other two during mid lactation. In the last two studies, the concentrate was pelleted and probably the rate of passage was higher than use fibrous by-products without pelleting. By other hand, fat added was another cause of the lack of effect observed in these last two trials; experiment 1 did not include added fat. Besides, the energy and protein of the diets in experiment 1 were lower than in the experiment 2 and 3. Therefore, evaluate the effect of low and high starch or the replacement of cereal grain with fibrous by-products on methane emission, was not useful when diets were balanced because it was not possible to isolate the ingredient or nutrient effect to evaluate. However, under practical or field condition these diets were useful because it was possible to replace cereal grain with fibrous by-product without detrimental effect on milk performance.
In spite of there are few reports in goats, most of the literature information available is *in vitro*, isolating the ingredient or additive to test, however in our studies the measurement were *in vivo* with diets similar to the field conditions. Besides, in our thesis in goats at mid or late lactation the ratio forage to concentrate was low, being more difficult to detect differences, under commercial conditions, between types of carbohydrates. Therefore, in this thesis the potential for reducing CH₄ through nutrition was modest.
The conclusions of the nutrition effect on methane emissions and performance in Murciano-Granadina goats are:

1. Mobile open circuit respiration calorimeter was an accurate technique to measure CH$_4$ and heat production, although comparison of face mask and head hood was not evaluated.
2. The average value of metabolizable energy intake of Murciano-Granadina goats during lactation was 1279 kJ/kg of BW$^{0.75}$ on average.
3. The efficiency use of metabolizable energy for milk production was 0.6 on average.
4. The replacement of cereal grain (from 30 to 60%) with fibrous by-products improve the percentage of milk fat in 1 point.
5. Replacing corn grain with beet pulp in the diet reduces the CH$_4$ production 10 g/day in Experiment 1.
6. Increasing the level of starch in the diet did not affect CH$_4$ production in Experiment 2 with average values of 28.5 g/day.
7. Substitution of barley grain with orange pulp or soy hulls did not influence CH$_4$ production (41 g/day on average).
8. Added fat to diets (fibrous by-product diets) in Experiments 2 and 3 could be the responsible of the lack of increase in CH$_4$ emissions in fibrous diets.
9. The CH$_4$ energy loss was 5% of the gross energy intake on average.


Kong, Y., He, M., McAlister, T., Seviour, R., Forster, R. (2010) Quantitative fluorescence in situ hybridization of microbial communities in the rumens of
cattle fed different diets. *Applied and Environmental Microbiology* 76, 6933-6938.


