UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Departamento de

Ciencia Animal

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

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- December 2015 -

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Influence of nutrition on methane gas production in Murciano-Granadina goats

151 pages

PhD Thesis, Universitat Politècnica de València, Valencia, Spain (2015)

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I would like to express my deepest gratitude to Carlos Fernández, for the continuous support of my PhD study and research, for his motivation and immense knowledge. Having my job outside of this university has made it challenging for us, thank you very much for your patience. I could not imagined having a better mentor for my PhD.

Special thanks goes to Mª Carmen López, Patricia Criscioni and Paula Monllor for helping me to carry out my experiments in the farm and laboratories. It would not have been possible without you. We made a good goat team with funny moments to remember.

I would like to thank Vicente Javier Moya, Luis Ródenas and Eugenio Martínez who were always willing to help and give their best advice. Thanks for providing me with an excellent atmosphere in the laboratory for learning and doing research.

Last but not least, my very sincere thanks to my friends, my sister and my parents for their love, ongoing support and for always being there.

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ADF	Acid detergent fiber
BHBA	ß-hydroxybutyrate
BP	Beet pulp diet
BRL	Barley diet
BW	Body weight
CB	Carbon balance
CH_4	Methane
C	Carbon
C_{CH4}	Carbon in CH_4
C_{CO2}	Carbon in CO_2
C_{feces}	Carbon in feces
C_{intake}	Carbon intake
C_{milk}	Carbon in milk
C_{urine}	Carbon in urine
CO_2	Carbon dioxide
CO_{2x}	Carbon dioxide production from oxidation
CORN	Diet corn
CP	Crude protein
DEI	Digestible energy intake
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
E_{feces}	Energy retained in feces
$E_{methane}$	Energy retained in methane
E_{milk}	Energy retained in milk
E_{urine}	Energy retained in urine
GE	Gross energy
GEI	Gross energy intake
H_2	Dihydrogen
HP	Heat production
HP_{CN}	Heat production determined by CN method
HPf	Heat production of fermentation
HPx	Heat production from oxidation of nutrients
H_3PO_4	Phosphoric acid
HS	High starch diet
H_2SO_4	Sulfuric acid
IC	Indirect calorimetry
k_l	Efficiency of use of ME for milk production
LS	Low starch diet
ME	Metabolizable energy
MEI	Metabolizable energy intake
N	Nitrogen
N_{feces}	Nitrogen in feces

N_{intake}	Nitrogen intake
N_{milk}	Nitrogen in milk
N_{urine}	Nitrogen in urine
NB	Nitrogen balance
NDF	Neutral detergent fiber
NEL	Net energy for lactation
NEFA	Non esterified fatty acids
NFC	Non fibrous carbohydrates
NH_3 N	Ammonia nitrogen
N_2O	Nitrous oxide
O_2	Oxygen
OH	Hydroxil radical
OM	Organic matter
OMI	Organic matter intake
OP	Orange pulp diet
OXCHO	Oxidation of carbohydrates
OXF	Oxidation of fat
OXP	Oxidation of protein
RE	Retained energy
RE_{body}	Retained energy in body tissues
RE_{fat}	Retained energy as fat
RE_{milk}	Retained energy as milk
$RE_{protein}$	Retained energy as protein
Re_{total}	$RE_{protein}$ + RE_{fat}
RQnpx	Non protein respiratory quotient from oxidation of nutrients
SEM	Standard error of the mean
SF_6	Sulphur hexafluoride
SH	Soybean hulls diet
T	Million tonne
TE	Tissue energy
VFA	Volatile fatty acids
Ym	Methane energy/gross energy intake

ABSTRACT - RESUMEN - RESUM

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

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 O_2, CO_2 and CH_4 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 O_2, CO_2 and CH_4 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 $BW^{0.75}$ 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 antropogénicas 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 O_2, CO_2 y CH_4 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 O_2, CO_2 y CH_4 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, eleven 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 alliberat 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 alliberat 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

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'O_2, CO_2 i CH_4 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 introduïa tot el cap i un programa informàtic gravava automàticament les concentracions d'O_2, CO_2 i CH_4 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.

GENERAL INTRODUCTION AND OBJECTIVES

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.

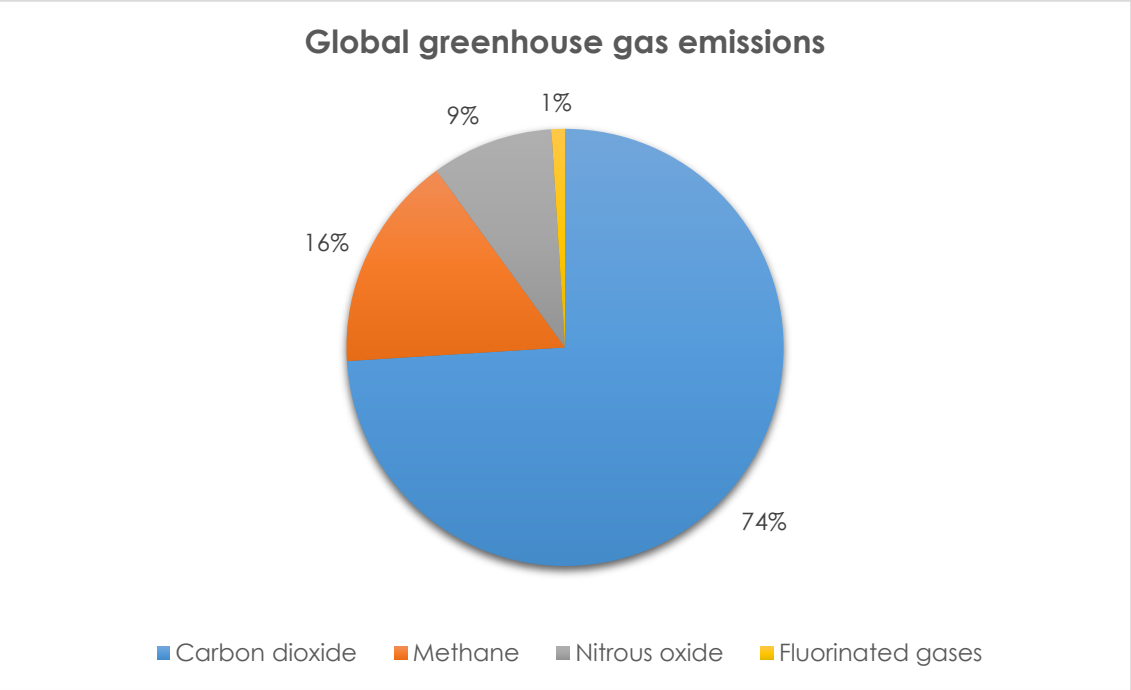
The principal forcing greenhouse gases are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and fluorinated gases (hydrofluorocarbons, perfluorocarbons and sulphur hexafluoride), and the main feedback greenhouse gas is water vapor.

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

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:



Source: Energy Sector Methane Recovery and Use: The Importance of Policy (2009). International Energy Agency.

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.

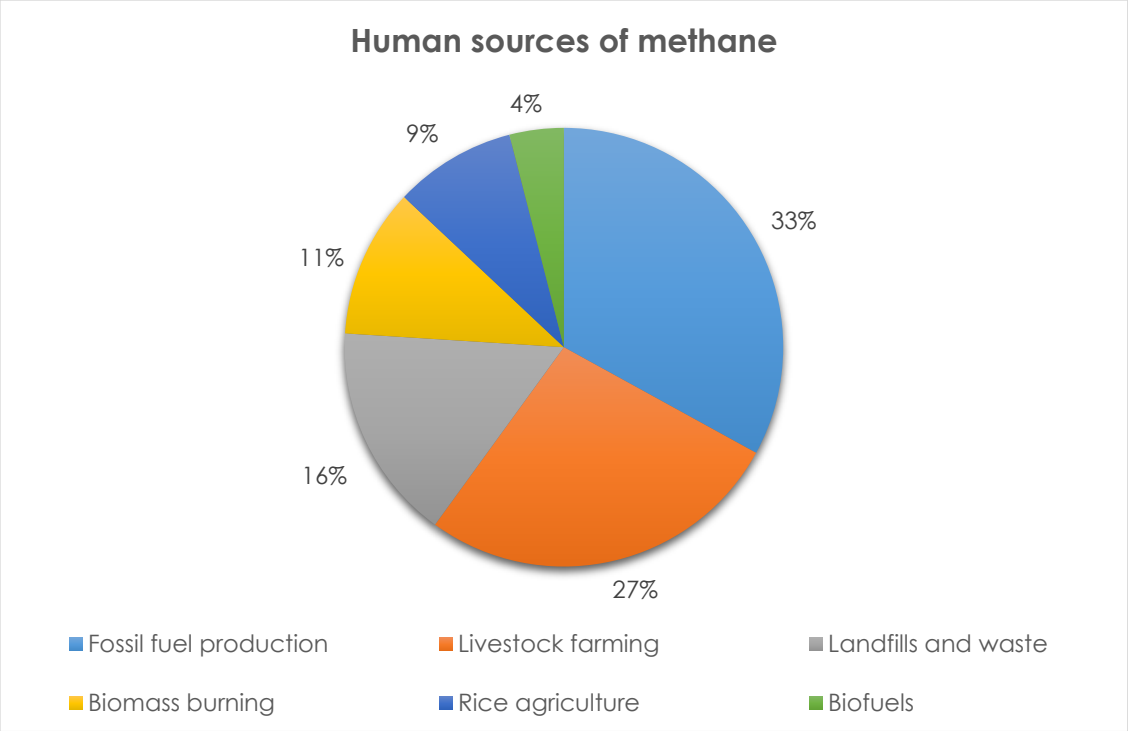
Methane levels have more than doubled over the last 150 years because of human activities like fossil fuel use and intensive farming. Before the Industrial Revolution, the atmospheric concentration of methane was maintained in a safe range by natural sinks. But for a long time new human activities have 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:



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, CO_2 and CH_4 (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:

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3\text{-}CH_2\text{-}COOH + 2H_2O$$

Butyric acid:

$$C_6H_{12}O_6 \rightarrow CH_3\text{-}CH_2\text{-}CH_2\text{-}COOH + 2CO_2 + 2H_2$$

$$4H_2 + CO_2 \rightarrow \mathbf{CH_4} + 2H_2O$$

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 flavefacience* can degrade cellulose and are thus referred to as cellulolytic 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 amylolyitca* 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 *Succiniclasticum 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 microorganims 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 3.5×109 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_4 emissions have been undertaken using indirect calorimetry techniques that calculated energy expenditure by the consumption of oxygen and expiration of CO_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_4 emissions in livestock. The predominant use of calorimeters has been to measure gaseous exchange as part of energy balance measurements, CH_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_4 in air entering and exiting, once adjusted for flow rate, allows the determination of CH_4 emissions. In contrast, in closed circuit systems air conditioning and CO_2 absorbents maintain the circulation of the air, the quality (humidity and temperature) and CO_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_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_2) and outputs (excretion, oxygen, CO_2 and CH_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_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_4 from ruminal and hindgut fermentations.

Disadvantages

While this technique is satisfactory for measuring CH_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:

$$F_{CH4} = F_{tracer} \times [(C_{measured\ CH4} - C_{atm\ CH4})/(C_{measured\ tracer} - C_{atm\ tracer})]$$

where F_{CH4} is the total production of CH_4, F_{tracer} is the total production or release of SF_6, $C_{measured\ CH4}$ and $C_{measured\ tracer}$ 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,

thereby diminishing the precision of calculated CH_4 yield (CH_4 expressed per unit of DMI).

The accuracy of the SF_6 technique to estimate CH_4 emissions is reliant on several assumptions, which are: that the SF_6 gas simulates the emissions of CH_4 and is uniformly mixed; dilution rates of SF_6 and CH_4 gases are identical; SF_6 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 SF_6 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 SF_6 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 green house 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 technic 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 then 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 methanogensisis (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 methanogensis (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 methanogensis. 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

Animal Production Science 55 (2014) 56-63. doi:10.1071/AN13119

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

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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 of 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 (O_2) consumption, carbon dioxide (CO_2), CH_4 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 CH_4 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

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 (20-23ºC) 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) H_2SO_4 to acidify the urine. Representative samples (20%) of diet, feces and urine were collected on 5 consecutive days, stored at -20ºC 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 4ºC 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% H_2SO_4 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

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 55ºC 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 ± 2ºC for 24 h and organic matter content (OM) was determined by incineration in an electric muffle furnace at 550ºC 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 a-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 (a-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% H_3PO_4 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

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 ºC. The initial oven temperature was set at 115 ºC held for 5 min and increased to 230 ºC at 8.5 ºC/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_{milk} (ARC 1980). The efficiency of use of ME for milk production (k_l) was calculated as E_{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:

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

The RE was calculated as the difference between MEI and HP. The body retention of energy (RE_{body}) was the difference between RE and milk energy (E_{milk}).

The energy associated with 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 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_2x) 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:

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

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

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

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

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 balance (g) × 6.25 × 23.86, and RE_{fat} = [C balance (g) - N balance (g) × 6.25 × 0.52] × 1.304 × 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: $HP_{CN} = MEI - TE_{total} - RE_{milk}$.

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 CH_4 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: $Y = \mu + D + T + D \times T + goat + \varepsilon$, 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 the 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 $P < 0.05$.

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

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) x 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

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 CH_4. The CH_4 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 CH_4 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 CH_4 production. However, Van Knegsel *et al.* (2007) found no differences in CH_4 production from dairy cows fed a diet contained 27% of corn with regards to a diet with 29% of BP. The measured CH_4 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_{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

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_l) 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/kg$^{0.75}$ BW). No significant differences were observed between diets for k_l (0.60 on average) and a similar value to that of Aguilera *et al.* (1990) was found with lactating Granadina goats (k_l=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 CH_4 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 CH_4 emission was significant ($P < 0.01$) and greater for BP than CORN (29.7 vs. 19.6 g/day). The CH_4 production in relation to ingested organic matter (OMI) reflected the differences in proportion between concentrates and roughages in the diets. The ratio CH_4 and OM intake was used as an indicator of fermentation level due to increase VFA production, more CO_2 is originated from fermentation processes and subsequently less from nutrient oxidation at the tissue level. The CH_4 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 Knegsel *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 CH_4 production. As we mentioned in Table 1.5, the CH_4 production increased from 19.6 to 29.7 g/day for diet CORN and BP, respectively. This increased in CH_4 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, CO_2 and milk C. Diet CORN had lower CH_4 than BP ($P < 0.05$) with values of 1.10 and 1.85 g/kg$^{0.75}$ BW, respectively. Values of N and C balances were converted to energy units and no significant differences were observed between diets for $RE_{protein}$, but diets tended ($P = 0.08$) to be different (57 vs. 32 kJ/kg$^{0.75}$ BW, in CORN and BP diets, respectively). Significant differences ($P < 0.02$) were found for RE_{fat} (300 vs. 44 kJ/kg$^{0.75}$ 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

This study was supported by INIA Project (ref. RTA2011-00107-C02-02).

1.7. 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 measure energy metabolism. *Archives of Animal Nutrition* 11, 1009-1018.

Aguilera, J.F., Prieto, C., Fonollá, J. (1990) Protein and energy metabolism of lactating Granadina goats. *British Journal of Nutrition* 63, 165-175. doi:10.1079/BJN19900104

AOAC International (2000) Official Methods of Analysis of the Association of Official Analytical Chemists, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

ARC (1980) The nutrient requirements of ruminant livestock, Technical Review. Farnham Royal. (CAB: Wallingford, UK).

Batey, I.L. (1982) Starch analysis using thermostable alpha-amylases. *Starch/Stärke* 34, 125-128.

Bava, L., Rapetti, L., Crovetto, G.M., Tamburini, A., Sandrucci, A., Galassi, G., Succi, G. (2001) Effect of a non forage diet on milk production, energy and nitrogen metabolism in dairy goats throughout lactation. *Journal of Dairy Science* 84, 2450-2459. doi:10.3168/jds.S0022-0302(01)74695-4

Bhattacharya, A.N., Lubbadah, W.F. (1971) Feeding high levels of beet pulp in high concentrate dairy rations. *Journal of Dairy Science* 54, 95-99. doi:10.3168/jds.S0022-0302(71)85784-3

Blaxter, K.L. (1967) Techniques in energy metabolism studies and their limitations. *The Proceedings of the Nutrition Society* 26, 86-96. doi:10.1079/PNS19670016

Brockway, J.M., Boyne, A.W., Gordon, J.G. (1971) Simultaneous calibration of gas analyzers and meters. *Journal of Applied Physiology* 31, 296-297.

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*

symposium on energy metabolism, Publication No. 8, Academic Press, London, 182-194.

Brouwer, E. (1965) Report of sub-committee on constants and factors. In: Blaxter, K.L., Ed., *Proceedings of the 3rd EAAP Symposium on energy metabolism*, Publication No. 11, Academic Press, London, 441-443.

Calsamiglia, S., Bach, A., de Blas, C., Fernández, C., García-Rebollar, P. (2009) Nutritional requirements for dairy ruminants. Fundación Española para el Desarrollo de la Nutrición Animal (FEDNA), Madrid.

Christensen, K., Chwalibog, A., Henckel, S., Thorbek, G. (1988) Heat production in growing pigs calculated according to the RQ and CN methods. *Comparative Biochemistry and Physiology* 91, 463468. doi:10.1016/0300-9629(88)90619-6

Chwalibog, A., Tauson, A.H., Thorbek, G. (1997) Quantitative oxidation of nutrients in growing calves. *Zeitschrift fur Ernahrungswissenschaft* 36, 313-316. doi:10.1007/BF01617806

Corbett, J.L., Freer, M. (2003) Past and present definitions of the energy and protein requirements of ruminants. *Asian-Australasian Journal of Animal Sciences* 16, 609-624. doi:10.5713/ajas.2003.609

European Union (2003) Protection of animals used for experimental purposes. Council Directive 86/609/EEC of 24 November 1986, amended 16.9.2003. European Council, Brussels, Belgium.

Fahey, G.C., Berger, L.L. (1988) Carbohydrate nutrition of ruminants. In 'The ruminant animal. Digestive nutrition and physiology'. (Ed. DC Church) pp. 269-297. (Prentice-Hall: Englewood Cliffs, NJ).

Fernández, C., López, M.C., Lachica, M. (2012*a*) Description and function of a mobile open-circuit respirometry system to measure gas exchange in small ruminants. *Animal Feed Science and Technology* 172, 242-246. doi:10.1016/j.anifeedsci.2012.01.006

Fernández, C., López, M.C., Lachica, M. (2012*b*) Heat production determined by the RQ and CN methods, fasting heat production and effect of the energy intake on substrates oxidation of indigenous Manchega sheep. *Animal Feed Science and Technology* 178, 115-119. doi:10.1016/j.anifeedsci.2012.09.007

Grainger, C., Beauchemin, K.A. (2011) Can enteric methane emissions from ruminants be lowered without lowering their production? *Animal Feed Science and Technology* 166-167, 308-320. doi:10.1016/j.anifeedsci.2011.04.021

Hindrichsen, I.K., Wettstein, H.R., Machmüller, A., Jörg, B., Kreuzer, M. (2005) Effect of the carbohydrate composition of feed concentrates on methane emission from dairy cows and their slurry. *Environmental Monitoring and Assessment* 107, 329-350. doi:10.1007/s10661-005-3008-3

Huntington, G.B. (1997) Starch utilization by ruminants: from basics to the bunk. *Journal of Animal Science* 75, 852-867.

Interal (2008) Estudio de Posicionamiento Estratégico para el sector de la Alimentación Animal en el escenario actual. Agrícola Española: Madrid.

Jouany, J.P. (1982) Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermentor contents. *Sciences des Aliments* 2, 131-144.

Kirkland, R.M., Gordon, F.J. (2001) The effect of milk yield and stage of lactation on the partitioning of nutrients in lactating dairy cows. *Journal of Dairy Science* 84, 233-240. doi:10.3168/jds.S0022-0302(01)74473-6

Lachica, M., Aguilera, J.F. (2003) Estimation of energy needs in the free-ranging goat with particular reference to the assessment of its energy expenditure by the 13C-bicarbonate method. *Small Ruminant Research* 49, 303-318. doi:10.1016/S0921-4488(03)00146-9

Mahjoubi, E., Amanlou, H., Zahmatkesh, D., Ghelich, M., Aghaziarati, N. (2009) Use of beet pulp as a replacement for barley grain to manage body

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

Mansfield, H.R., Stern, M.D., Otterby, D.E. (1994) Effect of beet pulp and animal byproducts on milk yield and in vitro fermentation by rumen microorganism. *Journal of Dairy Science* 77, 205-216. doi:10.3168/jds.S0022-0302(94)76943-5

McLean, J.A., Tobin, G. (1987) Animal and human calorimetry. Cambridge University Press, Cambridge.

NRC (2001) Nutrient requirements of dairy cattle. 7th rev. ed. National Academic Press, Washington, D. C.

Prieto, C., Aguilera, J.F., Lara, L., Fonollá, J. (1990) Protein and energy requirements for maintenance of indigenous Granadina goats. *The British Journal of Nutrition* 63, 155-163. doi:10.1079/BJN19900103

SAS (2001) Statistical Analysis System. User's guide. Version 8.02, Statistical Analysis System Institute Inc. Cary, NC, USA.

Tedeschi, L.O., Seo, S., Fox, D.G., Ruiz, R. (2006) Accounting for energy and protein reserves changes in predicting diet allowable milk production in cattle. *Journal of Dairy Science* 89, 4795-4807. doi:10.3168/jds.S0022-0302(06)72529-2

Tovar-Luna, I., Puchala, R., Sahlu, T., Freetly, H.C., Goetsch, A.L. (2010) Effects of stage of lactation and dietary concentrate level on energy utilization by Alpine dairy goats. *Journal of Dairy Science* 93, 4818-4828. doi:10.3168/jds.2010-3315

Van Knegsel, A.T.M., Van den Brand, H., Dijkstra, J., Van Straalen, W.M., Heetkamp, M.J., Tamminga, S., Kemp, B. (2007) Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. *Journal of Dairy Science* 90, 1467-1476. doi:10.3168/jds.S0022-0302(07) 71632-6

Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991) Methods for dairy fiber, neutral detergent, and non starch polysaccharides in relation to animal nutrition.

Journal of Dairy Science 74, 3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2

Voelker, J.A., Allen, M.S. (2003) Pelleted beet pulp substituted for high moisture corn. 1. Effects on feed intake, chewing behaviour and milk production of lactating dairy cows. *Journal of Dairy Science* 86, 3562-3570. doi:10.3168/jds.S0022-0302(03)73961-7

Waldo, D.R. (1973) Extent and partition of cereal grains starch digestion in ruminants. *Journal of Animal Science* 37, 1062-1074.

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

Ingredients (g/kg DM)	CORN[A]	BP
Alfalfa hay[B]	353	216
Barley straw	21	127
Corn	310	-
Barley	218	217
Beet pulp	-	302
Soy meal 44% crude protein	65	110
Calcium carbonate	15	13
Bicalcium phosphate	6	4
Sodium chloride	4	4
Vitamin mineral premix[C]	8	7
Chemical composition (% of DM)		
DM	88.1	88.5
Organic matter (OM = 100 - ash)	88.8	88.0
Crude Protein	14.7	15.7
Ether extract	2.7	1.9
Neutral detergent fiber	30.7	35.7
Acid detergent fiber	11.3	18.5
Starch	29.0	14.4
Carbon	41.3	41.7
NFC[D]	40.7	33.7
Sugar + pectins[E]	11.4	19.3
Gross energy (MJ/kg DM)	17.4	17.5

[A]CORN = corn diet; BP = beet pulp diet; DM = dry matter; OM = organic matter.

[B]All ingredients were pelleted with exception of alfalfa hay and barley straw. Chemical composition shown whole mixed ration.

[C]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.

[D]NFC = 100 - (neutral detergent fiber + crude protein + ash + ether extract); non fibrous carbohydrate.

[E]Sugars + 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.

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

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

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).

Items (%)	CORN	BP	SEM	*P*-value
DM[A]	79.8	74.2	1.24	0.06
Organic matter	80.2	75.5	1.22	0.05
Crude protein	76.6	75.7	1.53	0.78
Ether extract	80.3	59.6	3.08	0.01
Neutral detergent fiber	58.9	46.4	1.86	0.05
Starch	98.1	99.6	0.92	0.89
Energy	78.5	73.3	1.33	0.07

CORN = corn diet; BP = beet pulp diet; SEM = standard error of the mean.
[A]DM = dry matter.

Table 1.4. Rumen parameters: ammonia nitrogen (NH_3 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.

Items	CORN	BP	SEM	*P*-value
NH_3 N[A] (mg/dL)	24.59	12.69	4.46	0.04
Volatile Fatty Acids (mol/100 mol)				
Acetic	62.9	68.7	0.79	0.01
Propionic	15.3	13.1	1.03	0.15
Isobutyric	2.4	3.7	0.17	0.44
Butyric	15.1	9.8	0.25	0.08
Isovaleric	2.9	3.5	0.26	0.25
N-valeric	1.3	1.0	0.06	0.09
N-caproic	0.1	0.1	0.03	0.58

CORN = corn diet; BP = beet pulp diet; SEM = standard error of the mean.
[A]NH_3 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.

Items[A]	CORN	BP	SEM	*P*-value
GEI	1871	1595	84.46	0.05
E_{feces}	434	411	39.19	0.71
E_{urine}	56	48	2.53	0.11
$E_{methane}$	61	92	7.84	0.01
MEI	1320	1044	59.35	0.02
HP	738	691	37.05	0.44
RE	582	353	55.16	0.04
E_{milk}	277	292	19.96	0.12
RE_{body}	305	61	15.26	0.05
k_l	0.65	0.55	0.05	0.76
NE_L (MJ/kg DM)	8.2	6.5	0.08	0.03
CH_4 (g/day)	19.6	29.7	1.97	0.01
CH_4/OMI (g/kg)	12	22	2.01	0.01

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_{body} = MEI - HP - E_{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.

Items[A]	CORN	BP	SEM	*P*-value
HPx	718	675	37.20	0.34
OXP	61	89	8.52	0.10
OXCHO	384	257	54.38	0.05
OXF	274	329	32.18	0.10
OXP/HPx	9	14	1.65	0.06
OXCHO/HPx	48	37	6.35	0.04
OXF/HPx	43	49	5.82	0.06
RQnpx	0.97	0.81	0.01	0.12

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$^{0.75}$ BW), retained energy (RE; kJ/kg$^{0.75}$ BW) and heat production (HP; kJ/kg$^{0.75}$ BW) of Murciano-Granadina goats (n = 24) during late lactation according to the type of diet.

Items[A]	CORN	BP	SEM	*P*-value
N_{intake}	2.53	2.43	0.11	0.37
N_{feces}	0.59	0.58	0.06	0.88
N_{urine}	1.05	1.14	0.07	0.11
N_{milk}	0.51	0.51	0.03	0.98
NB	0.38	0.21	0.10	0.06
$RE_{protein}$ (kJ/ kg$^{0.75}$ BW)	57	32	4.39	0.08
C_{intake}	44.44	37.98	1.88	0.12
C_{feces}	10.68	10.01	0.97	0.74
C_{urine}	1.37	1.57	0.10	0.35
C_{milk}	5.44	5.81	0.44	0.71
C_{CO2}	18.82	17.20	0.94	0.75
C_{CH4}	1.10	1.85	0.14	0.01
CB	7.03	1.54	1.17	0.03
RE_{fat} (kJ/ kg$^{0.75}$ BW)	300	44	12.33	0.02
RE_{total} (kJ/ kg$^{0.75}$ BW)	357	76	19.64	0.02
RE_{milk} (kJ/ kg$^{0.75}$ BW)	272	291	22.07	0.32
HP_{CN} (kJ/ kg$^{0.75}$ BW)	691	677	47.52	0.54

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

[A]N = nitrogen; NB = nitrogen balance; RE = retention of energy; BW =body weight; C = carbon; CB = carbon balance; $RE_{total} = RE_{protein} + RE_{fat}$; $RE_{milk} = 51.8gC_{milk} - 19.38gN_{milk}$ (according to McLean and Tobin 1987); HP = heat production; $HP_{CN} = MEI - RE_{total} - RE_{milk}$.

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

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Open Journal of Animal Sciences 5 (2015) 198-209. doi: 10.4236/ojas.2015.52022

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

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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.*

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 problem, 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 green house 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 desviation, 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

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) H_2SO_4. 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% H_2SO_4 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,

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 N_2, CO_2 and CH_4 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 O_2, CO_2 and CH_4, respectively. 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 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), CH_4 production was maximized by diluting samples (1:9 ratio) to avoid any inhibition by excessive NH_3-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 N_2 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 CH_4 in biogas was determined by gas chromatography. An Agilent gas chromatograph, model 7890A coupled to a flame ionization detector (FID) and a HT3 Teledyne Tekmarhead-spaceautosampler (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,

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 CH_4 (with an energy equivalent value of 39.5 kJ/l CH_4, Brouwer, 1965). The heat production (HP) was calculated according to Brouwer (1965) for O_2 consumption (l/d), CO_2 and CH_4 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 (TE_{body}) was the difference between RE and milk energy (E_{milk}). 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 CH_4 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: $Y = \mu + D + T +$

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 CH_4 emissions was calculated by the following equations:

$$VCH_{4(i)} = (nRT)/P \quad \text{(E1)}$$

$$Bo = \Sigma VCH_{4(i)}/OM_{slurry} \quad \text{(E2)}$$

where n is the CH_4 mol amount in headspace, R is the ideal gas constant (atm $L \cdot K^{-1} \cdot mol^{-1}$), T is the incubating temperature in Kelvin (K), P is the pressure at the current measurement (atm), $VCH_{4(i)}$ is the volume of CH_4 in biogas at the current measurement (L), OM_{slurry} is the total amount of organic matter in dry weight basis in stored slurry (kg).

CH_4 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 ($P <$

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_{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.

2.4.3. Manure CH_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_4 production of goat feces as function of time during 42 days incubation is shown in Figure 1. The CH_4 production from HS (5.9 L CH_4/kg OM) treatment was higher ($P < 0.05$) compared to LS treatment (0.28 L CH_4/kg OM). The peak of CH_4 production in HS treatment took place 20 days after incubation started. For LS treatment, CH_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

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_{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_{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

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_{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 (Corbertt 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

(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 (Bo) 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

Aguerre, M.J., Mattiaux, M.A., Powell, J.M., Aguerre, A.F., Wattiaux, M.A., Powell, J.M. (2012) Emissions of ammonia, nitrous oxide, methane, and carbon dioxide during storage of dairy cow manure as affected by dietary forage-to-concentrate ratio and crust formation. *Journal of Dairy Science* 95, 7409-7416. doi:10.3168/jds.2012-5340

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

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

Aguilera, J.F., Prieto, C., Fonollá, J. (1990) Protein and energy metabolism of lactating Granadina goats. *British Journal of Nutrition* 63, 165-175. doi:10.1079/BJN19900104

AOAC International (2000) Official Methods of Analysis of the Association of Official Analytical Chemists, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Batey, I.L. (1982) Starch analysis using thermostable alpha-amylases. *Starch/Stärke* 34, 125-128. doi:10.1002/star.19820340407

Bava, L., Rapetti, L., Crovetto, G.M., Tamburini, A., Sandrucci, A., Galassi, G., Succi, G. (2001) Effect of a non forage diet on milk production, energy and nitrogen metabolism in dairy goats throughout lactation. *Journal of Dairy Science* 84, 2450-2459. doi:10.3168/jds.S0022-0302(01)74695-4

Beauchemin, K., McAllister, T.,McGinn, S. (2009) Dietary mitigation of enteric methane from cattle. CAB reviews: perspectives in agriculture, veterinary science. *Nutrition and Natural Resources* 4, 1-18.

Brockway, J.M., Boyne, A.W., Gordon, J.G. (1971) Simultaneous calibration of gas analyzers and meters. *Journal of Applied Physiology* 31, 296-297.

Brouwer, E. (1965) Report of sub-committee on constants and factors. In: Blaxter, K.L., Ed., *Proceedings of the 3rd EAAP Symposium on energy metabolism*, Publication No. 11, Academic Press, London, 441-443.

Calsamiglia, S., Bach, A., de Blas, C., Fernández, C., García-Rebollar, P. (2009) Nutritional requirements for dairy ruminants. Fundación Española para el Desarrollo de la Nutrición Animal (FEDNA), Madrid.

Casper, D.P., Maiga, H.A., Brouk, M.J., Schingoethe, D.J. (1999) Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. *Journal of Dairy Science* 82, 1779-1790. doi:10.3168/jds.S0022-0302(99)75408-1

Corbertt, J.L., Freer, M. (2003) Past and present definitions of the energy and protein requirements of ruminants. *Asian-Australasian Journal of Animal Sciences* 16, 609-624. doi:10.5713/ajas.2003.609

European Union (2003) Protection of animals used for experimental purposes. Council Directive 86/609/EEC of 24 November 1986, amended 16.9.2003. European Council, Brussels, Belgium.

Fernández, C., López, M.C., Lachica, M. (2012) Description and function of a mobile open-circuit respirometry system to measure gas exchange in small ruminants. *Animal Feed Science and Technology* 172, 242-246. doi:10.1016/j.anifeedsci.2012.01.006

Fernández, C., López, M.C., Lachica, M. (2015) Low-cost mobile open-circuit hood system for measuring gas exchange in small ruminants: from manual to automatic recording. *Journal of Agricultural Science*, 153, 1302-1309. doi:10.1017/S0021859615000416

Gerber, P.J., Hristov, A.N., Henderson, B., Makkar, H., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan, A.T., Yang, W.Z., Tricarico, J.M., Kebreab, E., Waghorn, G., Dijkstra, J., Oosting, S. (2013) Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. *Animal* 7, 220-234. doi:10.1017/S1751731113000876

Hindrichsen, I.K., Wettstein, H.R., Machmuller, A., Kreuzer, M. (2006) Methane emissions, nutrient degradation and nitrogen turnover in dairy cows and their slurry at different milk production scenarios with and without concentrate supplementation. *Agriculture, Ecosystems & Environment* 113, 150-161. doi:10.1016/j.agee.2005.09.004

Hoover, W.H., Stokes, S.R. (1991) Balancing carbohydrates and proteins for optimum rumen microbial yield. *Journal of Dairy Science* 74, 3630-3644. doi:10.3168/jds.S0022-0302(91)78553-6

Johnson, K.A., Johnson, D.E. (1995) Methane emissions in cattle. *Journal of Animal Science* 73, 2483-2492.

Jouany, J.P. (1982) Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermentor contents. *Sciences des Aliments* 2, 131-144.

Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P., Tricarico, J.M. (2014) Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions. *Journal of Dairy Science* 97, 3231-3261. doi:10.3168/jds.2013-7234

Kreuzer, M., Hindrichsen, I.K. (2006) Methane mitigation in ruminants by dietary means: the role of their methane emissions from manure. *International Congress Series* 1293, 199-208. doi:10.1016/j.ics.2006.01.015

Lachica, M., Aguilera, J.F. (2003) Estimation of energy needs in the free-ranging goat with particular reference to the assessment of its energy expenditure by the 13c-bicarbonate method. *Small Ruminant Research* 49, 303-318. doi:10.1016/S0921-4488(03)00146-9

Mathot, M., Decruyenaere, V., Stilmant, D., Lambert, R. (2012) Effect of cattle diet and manure storage conditions on carbon dioxide, methane and nitrous oxide emissions from tie-stall barns and stored solid manure. *Agriculture, Ecosystems & Environment* 148, 134-144. doi:10.1016/j.agee.2011.11.012

McLean, J.A., Tobin, G. (1987) Animal and human calorimetry. Cambridge University Press, Cambridge.

NRC (2001) Nutrient requirements of dairy cattle. 7th rev. ed. National Academic Press, Washington, D. C.

Orrico, M.A.P., Orrico, A.C.A., de Lucas, J., Sampaio, A.A.M., Fernandes, A.R.M., de Oliveira, D.A. (2012) Anaerobic biodigestion of beef cattle manure: influence of period, genotype and diet. *Brazilian Journal of Animal Science* 41, 1533-1538.

SAS (2001) Statistical Analysis System. User's guide. Version 8.02, Statistical Analysis System Institute Inc. Cary, NC, USA.

Triolo, J.M., Sommer, S.G., Moller, H.B., Weisbjerg, M.R., Jiang, X.Y. (2011) A new algorithm to characterize biodegradability of biomass during anaerobic digestion: influence of lignin concentration on methane production potential. *Bioresource Technology* 102, 9395-9402. doi:10.1016/j.biortech. 2011.07.026

Van Knegsel, A.T.M., Brand, H., Dijkstra, J., Straalen, W.M., Heetkamp, M.J.W., Tamminga, S., Kemp, B. (2007) Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. *Journal of Dairy Science* 90, 1467-1476. doi:10.3168/jds.S0022-0302(07)71632-6

Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991) Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2

Vedrenne, F., Véline, F., Dabert, P., Bernet, N. (2008) The effect of incubation conditions on the laboratory measurement of the methane producing capacity of livestock wastes. *Bioresource Technology* 99, 146-155. doi:10.1016/j.biortech.2006.11.043

Waldo, D.R. (1973) Extent and partition of cereal grains starch digestion in ruminants. *Journal of Animal Science* 37, 1062-1074.

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

Ingredients, g/kg DM	HS[A]	LS
Alfalfa hay	413	415
Barley	364	-
Soy meal 44% crude protein	155	100
Soy hulls	-	271
Gluten feed 18% crude protein	-	175
Lard[B]	19	19
By-pass fat[C]	6	12
Beet molasses	23	3
Calcium carbonate	8	2
Bicalcium phosphate	6	-
Sodium chloride	2.4	0.4
Premix[D]	2.6	2.6
Chemical composition, %		
Dry matter	87.5	88.3
Organic matter	91.8	91.5
Crude Protein	18.2	18.0
Ether extract	4.3	5.3
Neutral detergent fiber	40.6	46.5
Acid detergent fiber	19.1	27.0
NFC[E]	28.7	21.8
Starch	21.9	7.0
Gross energy, MJ/kg DM	19.0	19.2

[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.

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

Items[A]	HS	LS	SEM	*P*-value
Intake				
DMI, kg/d	2.03	2.07	0.040	NS
Digestibility				
DM, %	68	65	0.6	0.01
OM, %	70	66	0.7	0.01
CP, %	78	76	0.8	0.02
EE, %	84	86	0.5	0.07
NDF, %	47	48	0.8	NS
ADF, %	19	41	2.8	0.01
Starch, %	100	100	0.1	NS
GE, %	69	67	0.7	0.03

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).

Items[A]	HS	LS	SEM	*P*-value
Milk yield, kg/goat/day	2.4	2.2	0.07	0.01
DM, %	14.9	15.8	0.23	0.01
Fat, %	5.5	6.4	0.17	0.01
Protein, %	3.9	3.9	0.08	NS
Lactose, %	4.7	4.7	0.06	NS

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

Items[A]	HS	LS	SEM	*P*-value
pH	6.8	6.9	0.01	0.05
NH_3 N	18.2	25.4	4.37	0.05
Total VFA, meq/l	26.6	24.8	0.76	NS
Acetic	56.6	66.4	0.69	0.01
Propionic	21.4	18.0	0.93	0.01
Isobutyric	4.0	2.5	0.17	0.03
Butyric	8.5	12.9	0.25	0.01
Isovaleric	5.6	3.2	0.06	0.03
N-valeric	3.3	1.5	0.06	0.01
N-caproic	0.3	0.4	0.02	0.03

HS = high starch diet; LS = low starch diet; SEM = standard error of the mean.
[A]NH_3 N = ammonia nitrogen; VFA = volatile fatty acids; N = nitrogen.

Table 2.5. Energy balance in lactating goats (n = 20).

Items[A]	HS	LS	SEM	*P*-value
Energy balance, MJ/d				
GEI	38.4	39.8	0.76	NS
E_{feces}	12.0	13.3	0.45	0.02
DEI	26.4	26.5	0.44	NS
E_{urine}	1.2	0.8	0.16	NS
$E_{methane}$	1.6	1.6	0.03	NS
MEI	23.7	24.1	0.40	NS
HP	12.9	13.6	0.11	0.01
E_{milk}	9.1	8.9	0.24	NS
TE_{body}	1.7	1.5	0.41	NS

HS = high starch diet; LS =low starch diet; SEM = standard error of the mean.
[A]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).

Items[A]	HS	LS	SEM	*P*-value
Nitrogen balance, g/d				
N_{intake}	61.1	59.6	1.19	NS
N_{feces}	14.3	15.2	0.57	NS
N_{urine}	21.7	26.8	0.91	0.01
N_{milk}	14.2	13.2	0.32	NS
N recover	10.9	4.4	1.28	0.01

HS = high starch diet; LS =low starch diet; SEM = standard error of the mean.
[A]N = nitrogen.

Table 2.7. Enteric methane formation in lactating goats (n = 20).

Items[A]	HS	LS	SEM	*P*-value
CH_4, g/d	28.4	28.5	0.54	NS
CH_4/DMI, g/kg	14.1	13.8	0.36	NS
CH_4/OM intake, g/kg	15.4	15.1	0.39	NS
Ym, (CH_4/GEi)	4.2	4.0	0.11	NS
CH_4/milk, g/kg	11.7	13.2	0.42	0.03

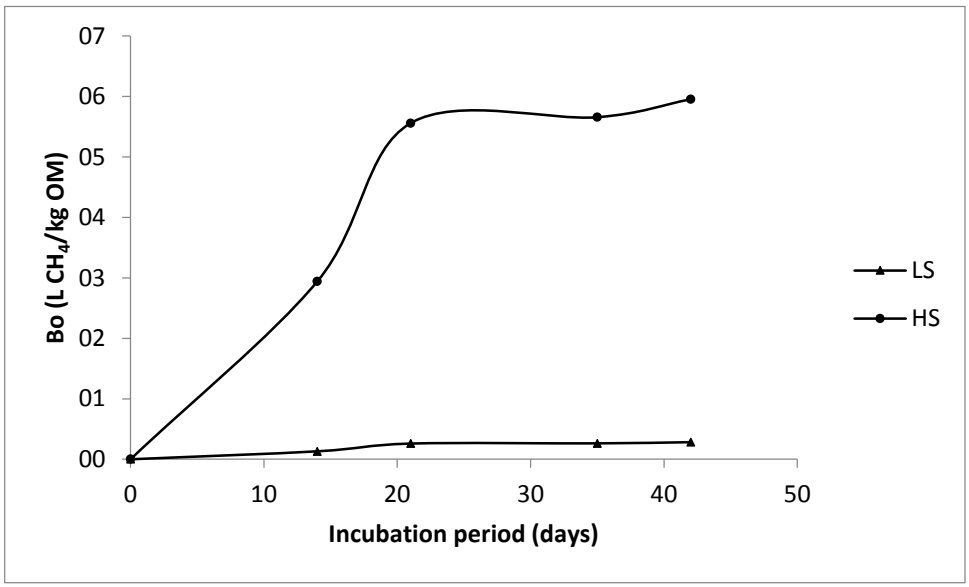
HS = high starch diet; LS =low starch diet; SEM = standard error of the mean.
[A]CH_4 = methane; DMI = dry matter intake; OM = organic matter; Ym = methane energy/gross energy intake; GEi = gross energy intake.

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

Items[A]	HS	LS	SEM	*P*-value
OM	0.20	0.25	0.011	0.02
CP	0.14	0.18	0.011	0.08
EE	0.10	0.11	0.006	NS
NDF	0.35	0.39	0.016	NS
ADF	0.52	0.43	0.021	0.02
Starch feces, mg/kg DM feces	0.50	0.20	0.010	NS

HS = high starch diet; LS =low starch diet; SEM = standard error of the mean.
[A]OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; DM = dry matter.

Figure 2.1. Cumulative methane production Bo (L CH_4/kg OM) from LS and HS diets in diluted goat feces during 42 days of incubation.



HS = high starch diet; LS =low starch diet; Bo = cumulative methane production; OM = organic matter.

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

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

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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 CH_4 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 ºC 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 H_2SO_4 to acidify the urine of each goat. The acidification of urine was necessary to prevent microbial degradation and the loss of volatile ammonia-N (NH_3-N). Representative samples (10%) of diet, feces and urine were collected over 5 consecutive days, stored at -20 ºC 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 vial 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_2SO_4 and frozen until later determination of NH_3-N. Samples for analysis of VFA were mixed with H_3PO_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).

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 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 lyophilization. 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 (Soxtec 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 (a-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/seg. 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.

NH_3-N 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 CH_4 (with an energy equivalent value of 39.5 kJ/L CH_4; Brouwer, 1965).

The heat production (HP) was determined from measurements of O_2 consumption, CO_2 and CH_4 production, and urine-N (N_{urine}), using the equation of Brouwer (1965):

$$HP\ (kJ) = (16.18 \times O_2) + (5.02 \times CO_2) - (2.17 \times CH_4) - (5.99 \times N_{urine})$$

where gases were expressed in liters per day and N_{urine} in grams per day. The body tissue energy (TE_{body}) was calculated as MEI - HP - milk energy (E_{milk}).

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 - ($CO_2/CH_4 \times CH_4$), according to Fahey and Berger (1988). The calculations were carried as follows:

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

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

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

Then, the HP from oxidation was:

$$HPx \text{ (kJ)} = (16.18 \times O_2) + (5.02 \times CO_{2x}) - (5.99 \times N_{urine})$$

Again, gases were expressed in liters per day and N_{urine} in grams per day. Heat of fermentation (HPf) was estimated subtracting HP from HPx. The non protein respiratory quotient from oxidation of nutrients (RQnpx) was determined as: $RQnpx = [CO_{2x} - (N_{urine} \times 6.25 \times 0.774)] / [O_2 - (N_{urine} \times 6.25 \times 0.957)]$.

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_{milk} + (0.84 x negative energy retention) + (1.05 x positive energy retention). The efficiency of use of ME for milk production (k_l) was calculated as corrected milk energy/(ME - ME_m), with MEm 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

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; $Y = \mu + D + T + goat + DxT + \varepsilon$, 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

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.

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_{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_{milk}. The TE_{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_l), according to AFRC (1993), was calculated as E_{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_l (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 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. 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. The N balance was positive for all treatments. 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 $TE_{protein}$ was 64, 28.3 and 50 kJ/kg of $BW^{0.75}$ for BRL, OP and SH, respectively. Regarding TE_{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; -

42.82 kJ TE_{fat}/kg of $BW^{0.75}$ in OP and SH diet on average against 19.2 kJ TE_{fat}/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_4 emissions from goats. The average CH_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_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_4 than fermentation of soluble sugars, which in turn produce more CH_4 than fermentation of starch. The similar CH_4 production that we found in this work can be explained by the fact that greater NH_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_4/kg milk was observed in the

second period of the trail. No effect of interaction between diet and period was observed. No effects of CH_4 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 NH_3-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 NH_3 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 TE_{fat} shown in Table 3.6 was -40.60 and -45 kJ/ kg of $BW^{0.75}$ 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 NH_3-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 heptadecanioic 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).

3.7. References

Agricultural and Food Research Council (AFRC) (1993) Energy and protein requirements of ruminants. CAB International, Wallingford, UK.

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 measure energy metabolism. *Archives of Animal Nutrition* 11, 1009-1018.

Aguilera, J.F., Prieto, C., Fonollá. J. (1990) Protein and energy metabolism of lactating Granadina goats. *Britrish Journal and Nutrition* 63, 165-175.

AOAC International (2000) Official Methods of Analysis of the Association of Official Analytical Chemists, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Batey, I.L. (1982) Starch analysis using thermostable alpha-amylases. *Stach/Stärke* 34, 125-128.

Bava, L., Rapetti, L., Crovetto, G.M., Tamburini, A., Sandrucci, A., Galassi, G., Succi, G. (2001) Effect of a non forage diet on milk production, energy and nitrogen metabolism in dairy goats throughout lactation. *Journal of Dairy Science* 84, 2450-2459.

Brockway, J.M., Boyne, A.W., Gordon, J.G. (1971) Simultaneous calibration of gas analyzers and meters. *Journal of Applied Physiology* 31, 296-297.

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 symposium on energy metabolism,* Publication No. 8, Academic Press, London, 182-194.

Brouwer, E. (1965) Report of sub-committee on constants and factors. In: Blaxter, K.L., Ed., *Proceedings of the* 3rd *EAAP Symposium on energy metabolism*, Publication No. 11, Academic Press, London, 441-443.

Casper, D.P., Maiga, H.A., Brouk, M.J., Schingoethe, D.J. (1999) Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. *Journal of Dairy Science* 82, 1779-1790. doi:10.3168/jds.S0022-0302(99)75408-1

Chilliard, Y., Ferlay, A., Rouel, J., Lamberet, G. (2003) A review and nutritional and physiological factors affecting goat milk lipids synthesis and lipolysis. *Journal of Dairy Science* 86, 1751-1770.

Chwalibog, A., Tauson, A.H., Thorbek, G. (1997) Quantitative oxidation of nutrients in growing calves. *Zeitschrift fur Ernahrungswissenschaft* 36, 313-316. doi:10.1007/BF01617806

European Union (2003) Protection of animals used for experimental purposes. Council Directive 86/609/EEC of 24 November 1986, amended 16.9.2003. European Council, Brussels, Belgium.

Fahey, G.C., Berger, L.L. (1988) Carbohydrate nutrition of ruminants. Pages 269-297 in Church, D.C. (Ed.), The Ruminant Animal. Digestive Nutrition and Physiology. Prentice-Hall, Englewood Cliffs, NJ.

Fernández, C., López, M.C., Lachica, M. (2012) Description and function of a mobile open-circuit respirometry system to measure gas exchange in small ruminants. *Animal Feed Science and Technology* 172, 242-246. doi:10.1016/j.anifeedsci.2012.01.006

Fernández, C., López, M.C., Lachica, M. (2015) Low cost open-circuit hood system for measuring gas exchange in small ruminants: from manual to automatic recording. *Journal of Agricultural Science* 153, 1302-1309.

Fievez, V., Colman, E., Castro-Montolla, J.M., Stefanov, I., Vlaeminck, B. (2012) Milk odd- and branched-chain fatty acids as biomarkers of rumen function-An update. *Animal Feed Science and Technology* 172, 51-65.

FEDNA (2010) Fundación Española para el Desarrollo de la Nutrición Animal. Tablas FEDNA de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos. 3rd ed. FEDNA, Madrid, Spain.

Grasser, L.A., Fadel, J.G., Garnett, I., Depeters, E.J. (1995) Quantity and economic importance of nine selected by-products used in California dairy rations. Journal of Dairy Science 78, 962-971.

Johnson, K.A., Johnson, D.E. (1995) Methane emissions in cattle. *Journal of Animal Science* 73, 2483-2492.

Jorjong, S., Van Knegsel, A.T.M., Verwaeren, J., Bruckmaier, R.M., De Baets, B., Kemp, B., Fievez, V. (2015) Milk fatty acids as possible biomarkers to diagnose hyperketonemia in early lactation. Journal of Dairy Science 98, 5211-5221.

Jouany, J.P. (1982) Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermentor contents. *Sciences des Aliments* 2, 131-144.

Kebreab, E., Strathe, A.B., Dijkstra, J., Mills, J.A.N., Reynolds, C.K., Crompton, L.A., Yan, T., France, J. (2010) Energy and protein interactions and their effects on nitrogen excretion in dairy cows. In: *Symposium on energy and protein metabolism and nutrition*, Parma, Italy, 417-426.

Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P., Tricarico, J.M. (2014) Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions. *Journal of Dairy Science* 97, 3231-3261. doi:10.3168/jds.2013-7234

López, M.C., Fernández, C. (2013) Energy partitioning and substrate oxidation by Murciano-Granadina goats during mid lactation fed soy hulls and corn gluten feed blend as a replacement for corn grain. *Journal of Dairy Science* 96, 4542-4552.

McLean, J.A., Tobin, G. (1987) Animal and Human Calorimetry. Cambridge University Press, Cambridge.

Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing beakers or crucibles: collaborative study. *Journal of Association of Official Analytical Chemists International* 85, 1217-1240.

NRC (2001) Nutrient requirements of dairy cattle. 7th rev. ed. National Academic Press, Washington, D. C.

O´Fallon, J.V., Busboom, J.R., Nelson, M.L., Gaskins, C.T. (2007) A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *Journal of Animal Science* 85, 1511-1521.

Palmquist, D.L., Jenkins, T.C. (1980) Fat in lactation rations: review. *Journal of Dairy Science* 63, 1-14.

SAS (2001) Statistical Analysis System. User's guide. Version 8.02, Statistical Analysis System Institute Inc. Cary, NC, USA.

Spek, J.W., Dijstra, J., Van Duinkerken, G., Bannink, A. (2013) A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. *Journal of Agricultural Science* 151, 407-423.

Tovar-Luna, I., Puchala, R., Sahlu, T., Freetly, H.C., Goetsch, A.L. (2010) Effects of stage of lactation and dietary concentrate level on energy utilization by Alpine dairy goats. *Journal of Dairy Science* 93, 4818-4828. doi:10.3168/jds.2010-3315

Ulbricht, T.L., Southgate, D.A.T. (1991) Coronary Heart Disease: Seven Dietary Factors. *Lancet* 338, 985-992.

Van Knegsel, A.T.M., Brand, H., Dijkstra, J., Straalen, W.M., Heetkamp, M.J.W., Tamminga, S., Kemp, B. (2007) Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. *Journal of Dairy Science* 90, 1467-1476. doi:10.3168/jds.S0022-0302(07)71632-6

Vasta, V., Nudda, A., Cannas, A., Lanza, M., Priolo, A. (2008) Alternative feed resources and their effects on the quality of meat and milk from small ruminants. *Animal Feed Science and Technology* 147, 223-246.

Vlaeminck, B., Fievez, V., Cabrita, A.R.J., Fonseca, A.J.M., Dewhurst, R.J. (2006) Factors affecting odd-and branched-chain fatty acids in milk: a review. *Animal Feed Science and Technology* 131, 389-417.

Vlaeminck, B., Gervais, R., Rahman, M.M., Gadeyne, F., Gorniak, M., Doreau, M., Fievez, V. (2015) Postruminal synthesis modifies the odd- and branched-chain fatty acid profile from the duodenum to milk. *Journal of Dairy Science* 98, 4829-4840.

Table 3.1. Ingredients and chemical composition of the diets.

	Diet[A]		
Ingredients, g/kg DM[B]	BRL	OP	SH
Alfalfa hay	330.0	330.0	330.0
Barley	594.2	-	-
Orange pulp	-	593.7	-
Soy hulls	-	-	595.3
Soy meal 44% crude protein	46.8	59.3	39.6
Calcium carbonate	11.8	7.6	4.3
Sodium chloride	8.7	2.7	7.5
Bypass fat[C]	4.2	2.5	19.1
Premix[D]	4.2	4.2	4.3
Chemical composition, % of DM			
Dry matter	89.6	87.5	89.2
Organic matter	92.8	91.0	91.4
Crude protein	13.7	11.9	14.0
Ether extract	2.2	1.1	1.8
Neutral detergent fiber	41.5	31.4	54.6
Acid detergent fiber	15.8	20.8	38.2
NFC[E]	35.5	46.6	21.0
Starch	32.7	5.1	1.8
Gross energy, MJ/kg DM[E]	17.9	16.9	18.0

[A]BRL = barley; OP = orange pulp; SH = soybean hulls.

[B]DM = dry matter

[C]Bypass fat of palm fatty acid distillate. Provided by Norel Animal Nutrition, Norel S.A., Spain.

[D]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.

[E]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.

	Diet[1]			Period[2]		SEM[3]	*P-Value*		
Items[4]	BRL	OP	SH	1	2		Diet	Period	Diet x Period
BW, kg	42.4	42.3	41.6	42.2	42.2	0.95	0.949	0.786	0.505
DMI, kg/d	2.0	2.0	2.1	2.0	2.0	0.02	0.248	0.259	0.244
Digestibility, % of DM									
DM	71.6^{a}	73.5^{a}	64.0^{b}	70.3	69.7	0.93	0.000	0.579	0.871
OM	74.5^{b}	77.5^{a}	66.6^{c}	73.4	72.9	1.00	0.000	0.554	0.927
CP	65.6^{a}	56.4^{b}	59.3^{ab}	60.9	61.8	1.29	0.006	0.605	0.717
EE	74.2^{a}	34.0^{b}	71.1^{a}	60.0	64.8	4.36	0.000	0.365	0.568
NDF	64.0	61.4	60.8	63.6	61.2	0.75	0.145	0.117	0.509
ADF	47.9^{c}	65.6^{a}	58.7^{b}	55.6	55.7	1.78	0.000	0.964	0.920
Starch	99.4^{a}	98.2^{b}	91.9^{c}	97.0	97.0	0.70	0.000	0.886	0.041
GE	73.4^{a}	73.4^{a}	67.2^{b}	70.3	73.1	0.89	0.001	0.021	0.238

$^{a-c}$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.

	Diet[1]				*P-Value*
Items[3]	BRL	OP	SH	SEM[2]	Diet
pH	7.30^{a}	7.10^{b}	7.10^{b}	0.041	0.031
NH_3-N, mg/dL	15.08^{b}	52.30^{a}	38.33^{a}	7.405	0.039
Total VFA, mM	14.16^{c}	25.97^{a}	23.94^{b}	3.289	0.016
Individual VFA, mol/100 mol					
Acetic acid	62.22^{a}	56.98^{b}	61.48^{a}	1.435	0.031
Propionic acid	13.42	14.98	15.90	0.624	0.293
Isobutiric acid	3.86	2.87	3.19	0.362	0.591
Butyric acid	14.39^{b}	19.54^{a}	13.09^{b}	1.389	0.023
Isovaleric acid	4.34	3.81	3.74	0.422	0.859
N-Valeric acid	1.78	1.70	1.74	0.147	0.980
N-Caproic acid	0.00^{b}	0.12^{a}	0.62^{a}	0.103	0.004
Heptanoic acid	0.00^{b}	0.00^{b}	0.23^{a}	0.056	0.004

$^{a\text{-}c}$ 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]NH_3-N = ammonia nitrogen; VFA = volatile fatty acids; N = nitrogen.

Table 3.4. Daily energy partitioning (kJ/kg of $BW^{0.75}$) of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

	Diet[1]			Period[2]		SEM[3]	*P-Value*		
Items[4]	BRL	OP	SH	1	2		Diet	Period	Diet x Period
GEI	2170.2	2055.8	2296.2	2142.5	2205.6	42.66	0.113	0.451	0.243
E_{feces}	582.0^{b}	545.0^{b}	758.9^{a}	651.6	605.8	26.06	0.001	0.251	0.416
E_{urine}	40.4^{b}	56.7^{a}	69.0^{a}	50.3	60.4	5.24	0.041	0.344	0.916
$E_{methane}$	135.9	141.5	135.4	129.7	145.5	4.60	0.852	0.111	0.231
MEI	1411.7	1312.5	1333.0	1310.9	1393.9	29.51	0.301	0.167	0.177
HP	817.6	847.1	803.8	795.2^{b}	848.6^{a}	14.06	0.471	0.033	0.312
E_{milk}	513.5	474.6	527.8	572.0	492.3	14.65	0.473	0.052	0.027
TE_{body}	80.5^{a}	-9.2^{b}	1.3^{b}	-5.1^{b}	74.1^{a}	26.06	0.017	0.021	0.981

^{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]GEI = gross energy intake; E_{feces} = energy losses in feces; E_{urine} = energy losses in urine; $E_{methane}$ = energy losses in methane; MEI = metabolizable energy intake; HP = heat production; E_{milk} = milk energy; TE_{body} = recovered energy in tissue ($TE_{body} = MEI - HP - E_{milk}$).

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.

	Diet[1]			Period[2]		SEM[3]	*P-Value*		
Items[4]	BRL	OP	SH	1	2		Diet	Period	Diet x Period
HPx	795.7	811.7	759.7	765.6	815.0	13.47	0.458	0.033	0.329
HPf	21.9^{c}	35.4^{b}	44.1^{a}	29.7	33.6	2.32	0.000	0.268	0.442
OXP	56.0	47.5	66.4	49.1^{b}	63.9^{a}	3.45	0.083	0.014	0.489
OXCHO	471.9^{a}	363.0^{b}	115.6^{c}	329.0	361.0	35.65	0.000	0.123	0.219
OXF	267.7^{c}	401.1^{b}	577.5^{a}	387.3	389.9	33.23	0.000	0.505	0.148
OXP/HPx	7.1	5.8	8.8	6.4	7.9	0.44	0.056	0.054	0.733
OXCHO/HPx	59.2^{a}	45.0^{b}	14.6^{c}	42.1	44.3	4.28	0.000	0.266	0.178
OXF/HPx	33.7^{c}	49.2^{b}	76.6^{a}	51.5	47.8	4.40	0.000	0.175	0.186
RQnpx	0.89^{a}	0.84^{b}	0.76^{c}	0.82	0.84	0.01	0.000	0.191	0.142

$^{a\text{-}c}$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 {$[CO_{2x} - (N_{urine} \times 6.25 \times 0.774)]/[O_2 - (N_{urine} \times 6.25 \times 0.957)]$, where CO_2 = CO_2 production from oxidation and N_{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.

	Diet[1]			Period[2]		SEM[3]	*P-Value*		
Items[4]	BRL	OP	SH	1	2		Diet	Period	Diet x Period
C_{intake}	52.4	50.1	56.1	52.0	53.2	1.04	0.103	0.446	0.242
C_{feces}	15.0^{b}	13.7^{b}	19.8^{a}	15.7	16.3	0.70	0.001	0.369	0.555
C_{urine}	1.3^{b}	1.6^{b}	1.8^{b}	1.5	1.5	0.09	0.045	0.728	0.427
C_{CO2}	21.5	22.6	21.1	20.8^{b}	22.5^{a}	0.44	0.366	0.026	0.244
C_{CH4}	1.8	1.9	1.8	1.8	1.9	0.08	0.851	0.111	0.231
C_{milk}	11.1	10.5	11.4	11.6	10.4	0.30	0.408	0.089	0.027
$C_{retained\ body}$	1.8^{a}	0.2^{b}	-0.2^{c}	1.1^{a}	0.5^{b}	0.51	0.026	0.045	0.701
N_{intake}	2.7	2.3	2.9	2.6	2.6	0.06	0.003	0.460	0.238
N_{feces}	0.9	1.0	1.2	1.0	1.0	0.04	0.075	0.978	0.870
N_{urine}	0.5^{b}	0.4^{b}	0.6^{a}	0.4^{b}	0.6^{a}	0.03	0.043	0.014	0.498
N_{milk}	0.8	0.7	0.8	0.8	0.8	0.02	0.044	0.248	0.075
$N_{retained\ body}$	0.4^{a}	0.2^{b}	0.3^{a}	0.4	0.3	0.04	0.043	0.145	0.196
TE $_{protein}$, kJ/kg of $BW^{0.75}$	64.0^{a}	28.3^{c}	50.0^{b}	58.5	42.2	11.52	0.023	0.238	0.552
TE $_{fat}$, kJ/kg of $BW^{0.75}$	19.2^{a}	-40.6^{b}	-45.0^{b}	-9.3^{b}	-20.0^{a}	10.56	0.045	0.048	0.506

$^{a-c}$ 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]C_{intake} = C intake; C_{feces} = C losses in feces; C_{urine} = C losses in urine; C_{CO2} = C losses in CO_2; C_{CH4} = C losses in methane; C_{milk} = recovered C in milk; $C_{retained\ body}$ = recovered C in tissue; N_{intake} = N intake; N_{feces} = N losses in feces; N_{urine} = N losses in urine; N_{milk} = recovered N in milk; $N_{retained\ body}$ = recovered N in tissue; TE_{body} = recovered energy in tissue (TE_{body} = MEI − HP − E_{milk}).

Table 3.7. Daily milk production and composition of Murciano-Granadina goats (n = 12) during mid lactation according to the type of diet.

	Diet[1]			Period[2]		SEM[3]	*P-Value*		
Items	BRL	OP	SH	1	2		Diet	Period	Diet x Period
Milk yield, g/goat/day	2285.4	2013.8	2166.5	2379.2^{a}	1931.2^{b}	81.72	0.247	0.004	0.612
Composition, %									
DM[4]	14.7	15.7	15.4	14.5^{b}	16.0^{a}	0.28	0.296	0.010	0.553
Fat	5.4	6.3	6.4	5.7	6.3	0.21	0.073	0.121	0.602
Protein	4.0	3.7	3.7	3.3^{b}	4.2^{a}	0.13	0.544	0.000	0.226
Lactose	4.6	4.7	4.5	4.6	4.6	0.05	0.613	0.906	0.220

^{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]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.

	Diet[1]			SEM[2]	*P*-Value
Items[3]	BRL	OP	SH		
Butyric Acid	0.30	0.32	0.31	0.007	0.429
Caproic Acid	0.95	0.97	0.92	0.025	0.760
Caprylic Acid	1.82	1.74	1.68	0.050	0.539
Capric Acid	10.03	9.60	8.81	0.236	0.098
Undecanoic Acid	0.37	0.32	0.29	0.022	0.321
Lauric Acid	6.89	5.99	5.46	0.297	0.119
Myristic Acid	13.38	13.13	11.94	0.327	0.185
Myristoleic Acid	0.28	0.30	0.27	0.025	0.878
Pentadecanoic Acid	0.22^{a}	0.14^{b}	0.15^{b}	0.002	0.247
Palmitic Acid	40.61^{b}	44.53^{a}	42.63^{ab}	0.620	0.021
Palmitoleic Acid	1.11	1.36	1.27	0.074	0.391
Heptadecanoic Acid	0.59^{b}	0.70^{a}	0.71^{a}	0.023	0.039
cis-10-Heptadecenoic Acid	0.28^{b}	0.36^{a}	0.33^{ab}	0.015	0.047
Stearic Acid	4.69	3.77	4.96	0.324	0.375
Elaidic Acid	0.37^{a}	0.32^{a}	0.23^{b}	0.003	0.019
Oleic Acid	13.43^{b}	12.45^{b}	16.70^{a}	0.616	0.017
Vaccenic	0.31^{b}	0.46^{a}	0.41^{a}	0.018	0.000
Linoleic Acid	3.18^{a}	2.25^{b}	1.95^{b}	0.150	0.000
Arachidic Acid	0.11^{b}	0.10^{b}	0.16^{a}	0.006	0.000
gamma-Linolenic Acid	0.00	0.00	0.00	0.002	0.572
cis-11-Eicosenoic Acid	0.07^{a}	0.03^{c}	0.05^{b}	0.004	0.000
Linolenic Acid	0.35^{b}	0.52^{a}	0.34^{b}	0.027	0.008
CLA 9c11t + 9t11c	0.47^{a}	0.41^{ab}	0.28^{b}	0.027	0.006
CLA 10t12c	0.01^{c}	0.04^{a}	0.02^{b}	0.003	0.000
Arachidonic Acid	0.19^{a}	0.16^{ab}	0.13^{b}	0.010	0.036
Medium-chain fatty acids	20.04^{a}	18.62^{ab}	17.16^{b}	0.505	0.051
Monounsaturated fatty acids	15.84^{b}	15.30^{b}	19.25^{a}	0.603	0.013
Polyunsaturated fatty acids	4.22^{a}	3.38^{b}	2.72^{b}	0.187	0.001
Saturated fatty acids	79.94	81.32	78.02	0.586	0.106
AI	5.15	5.64	4.42	0.218	0.076

$^{a-c}$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]CLA = 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.

	Diet[1]			Period[2]			*P-Value*		
Items[4]	BRL	OP	SH	1	2	SEM[3]	Diet	Period	Diet x Period
CH_4, g/d	40.5	42.3	39.4	38.8	42.6	1.41	0.762	0.158	0.597
Ym, %	6.3	6.9	5.9	6.1	6.6	0.21	0.170	0.187	0.732
CH_4/DMi, g/kg	20.1	21.1	19.0	19.3	20.9	0.61	0.498	0.183	0.717
CH_4/OMi, g/kg	21.7	23.1	20.7	20.9	22.7	0.67	0.467	0.177	0.715
CH_4/milk, g/kg	18.3	21.4	18.4	16.2	22.1	0.91	0.131	0.000	0.928

[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.

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

$^{a-c}$ 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 CH_4 formation than mixed or concentrated based diets, as well high starch content in diet reduces the CH_4 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 CH_4 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 CH_4 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 CH_4 production. The higher ammonia N observed in the corn diet is the responsible of less fermentative activity and lower CH_4 production, too.

In the second experiment, the average CH_4 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 CH_4 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 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 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 CH_4 emission from enteric fermentation was similar between diets and no differences were

observed in Ym. However, the amount of 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 CH_4 emission in cattle ranged from 6% to 10%. Patra and Lalhriatpuii (2016) suggested that CH_4 production is lower in goats than in cattle due to grater passage rate of feeds for goats than for cattle, and consequently low 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 CH_4 through nutrition was modest.

CONCLUSIONS

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 efficicency 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.

REFERENCES

Asanuma, N., Iwamoto, M., Hino, T. (1999) The production of formate, a substrate for methanogenesis, from compounds related with the glyoxylate cycle by mixed ruminal microbes. *Animal Science Journal* 70, 67-73.

Beauchemin, K.A., Kreuzer, M., O'Mara, F., McAllister, T.A. (2008) Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture* 48, 21-27.

Beauchemin, K.A., McGinn, S.M. (2006) Methane emissions from beef cattle: effects of fumaric acid, essential oil, and canola oil. *Journal of Animal Science* 84, 1489-1496.

Beauchemin, K.A., McGinn, S.M., Benchaar, C., Holtshausen, L. (2009) Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. *Journal of Dairy Science* 92, 2118-2127.

Beauchemin, K.A., McGinn, S.M., Martinez, T.F., McAllister, T.A. (2007) Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. *Journal of Animal Science* 85, 1990-1996.

Beauchemin, K.A., McGinn, S.M., Petit, H.V. (2007) Methane abatement strategies for cattle: lipid supplementation of diets. *Canadian Journal of Animal Science* 87, 431-440.

Blaxter, K.L. (1989) Energy Metabolism in Animals and Man. Cambridge, UK Cambridge Univ. Press.

Boadi, D.A., Wittenberg, K.M., Kennedy, A.D. (2002) Validation of the sulphur hexafluoride (SF_6) tracer gas technique for measurement of methane and carbon dioxide production by cattle. *Canadian Journal of Animal Science* 82, 125-131.

Bousquet, P., Ciais, P., Miller, J.B., Dlugokencky, E.J., Hauglustaine, D.A., Prigent, C., Van der Werf, G.R., Peylin, P., Brunke, E.G., Carouge, C., Langenfelds, R.L., Lathière, J., Papa, F., Ramonet, M., Schmidt, M., Steele, L.P., Tyler, S.C., White,

J. (2006) Contribution of anthropogenic and natural sources to atmospheric methane variability. *Nature* 443, 439-443. doi:10.1038/nature05132

Burt, S. (2004) Essential oils: their antibacterial properties and potential applications in foods: a review. *International Journal of Food Microbiology* 94, 223-253.

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., Ferret, A. (2007) Invited review: essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science* 90, 2580-2595.

Carro, M.D. and Ranilla, M.J. (2003) Effect of the addition of malate on in vitro rumen fermentation of cereal grains. *British Journal of Nutrition* 89, 181-188.

Carulla, J.E., Kreuzer, M., Machmüller, A., Hess, H.D. (2005) Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Australian Journal of Agricultural Research* 56, 961-970.

Cosgrove, G.P., Waghorn, G.C., Anderson, C.B., Peters, J.S., Smith, A., Molano, G. (2008) The effect of oils fed to sheep on methane production and digestion of ryegrass pasture. *Australian Journal of Experimental Agriculture* 48, 189-192.

Dohme, F., Machmüller, A., Wasserfallen, A., Kreuzer, M. (2000) Comparative efficiency of various fats rich in medium-chain fatty acids to suppress ruminal methanogenesis as measured with RUSITEC. *Canadian Journal of Animal Science* 80, 473-482.

Dohme, F., Machmüller, A., Wasserfallen, A., Kreuzer, M. (2001) Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. *Letters in Applied Microbiology* 32, 47-51.

Ermler, U., Grabarse, W., Shima, S., Goubeaud, M., Thauer, R.K. (1997) Crystal structure of methyl coenzyme M reductase: the key enzyme of biological methane formation. *Science* 278,1457-1462.

Energy sector methane recovery and use: the importance of policy (2009). International Energy Agency.

Eugène, M., Massé, D., Chiquette, J., Benchaar, C. (2008) Meta-analysis on the effects of lipid supplementation on methane production in lactating dairy cows. *Canadian Journal of Animal Science* 88, 331-334.

Ferry, J.G. (1992) Methane from acetate. *Journal of Bacteriology* 174, 5489-5495.

Finlay, B.J., Esteban, G., Clarke, K.J., Williams, A.G., Embley, T.M., Hirt, R.P. (1994) Some rumen ciliates have endosymbiotic methanogens. *FEMS Microbiology Letters* 117, 157-161.

Foley, P.A., Kenny, D.A., Callan, J.J., Boland, T.M., O'Mara, F.P. (2009) Effect of DL-malic acid supplementation on feed intake, methane emission, and rumen fermentation in beef cattle. *Journal of Animal Science* 87, 1048-1057.

Grainger, C., Williams, R., Clarke, T., Wright, A.D.G., Eckard, R.J. (2010) Supplementation with whole cottonseed causes long-term reduction of methane emissions from lactating dairy cows offered a forage and cereal grain diet. *Journal of Dairy Science* 93, 2612-2619.

Greenhouse gas emissions (2015). United States Environmental Protection Agency. http://www.epa.gov/climatechange/ghgemissions/

Guo, Y.Q., Liu, J.X., Lu, Y., Zhu, W.Y., Denman, S.E., McSweeney, C.S. (2008) Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen micro-organisms. *Letters in Applied Microbiology* 47, 421-426.

Hegarty, R.S. (1999) Reducing rumen methane emissions through elimination of rumen protozoa. *Australian Journal of Agricultural Research* 50, 1321-1327.

Hindrichsen, I.K., Wettstein, H.R., Machmüller, A., Kreuzer, M. (2006) Methane emission, nutrient degradation and nitrogen turnover in dairy cows and their slurry at different milk production scenarios with and without concentrate supplementation. *Agriculture, Ecosystems and Environment* 113, 150-161.

Hobson, P.N., Stewart, C.S. (1997) The Rumen Microbial Ecosystem, Blackie Acad. Profess. London.

Holter, J.B., Young, A.J. (1992) Methane prediction in dry and lactating Holstein cows. *Journal of Dairy Science* 75, 2165-2175.

Holtshausen, L., Chaves, A.V., Beauchemin, K.A., McGinn, S.M., McAllister, T.A., Odongo, N.E., Cheeke, P.R., Benchaar, C. (2009) Feeding saponin-containing *Yucca schidigera* and *Quillaja saponaria* to decrease enteric methane production in dairy cows. *Journal of Dairy Science* 92, 2809-2821. doi:10.3168/jds.2008-1843

Hungate, R. E., Smith, W., Bauchop, T., Ida Yu, Rabinowitz, J.C. (1970) Formate as an intermediate in the bovine rumen fermentation. *Journal of Bacteriology* 102, 389-397.

Jarvis, G.N., Strompl, C., Burgess, D.M., Skillman, L.C., Moore, E.R.B., Joblin, K.N. (2000) Isolation and identification of ruminal methanogens from grazing cattle. *Current Microbiology* 40, 327-332.

Johnson, K., Huyler, M., Westberg, H., Lamb, B., Zimmerman, P. (1994) Measurement of methane emissions from ruminant livestock using a sulfur hexafluoride tracer technique. *Environmental Science and Technology* 28, 359-362. doi:10.1021/es00051a025

Johnson, K.A., Johnson, D.E. (1995) Methane emissions in cattle. *Journal of Animal Science* 73, 2483-2492.

Johnson, D.E., Ward, G.M. (1996) Estimates of animal methane emissions. *Environmental Monitoring and Assessment* 42, 133-141.

Jordan, E., Kenny, D., Hawkins, M., Malone, R., Lovett, D.K., O'Mara, F.P. (2006) Effect of refined soy oil or whole soybeans on intake, methane output, and performance of young bulls. *Journal of Animal Science* 84, 2418-2425.

Jordan, E., Lovett, D.K., Monahan, F.J., Callan, J., Flynn, B., O'Mara, F.P. (2006) Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers. *Journal of Animal Science* 84, 162-170.

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.

Lassey, K.R., Ulyatt, M.J., Martin, R.J., Walker, C.F., Shelton, I.D. (1997) Methane emissions measured directly from grazing livestock in New Zealand. *Atmospheric Environment Volume* 31, 2905-2914. doi:10.1016/S1352-2310(97)00123-4

Lassey, K.R., Walker, C.F., McMillan, A.M.S., Ulyatt. M.J. (2001) On the performance of SF_6 permeation tubes used in determining methane emission from grazing livestock. *Chemosphere Global Change Science* 3, 367-376.

Liang, J. B., Terada, F., Hamaguch, I. (1989) Efficacy of using the face mask technique for the estimation of daily heat production of cattle. In: Energy metabolism of farm animals (Ed. Y. Van Der Honing and W. H. Close). Pudoc, Wageningen, the Netherlands.

Machmüller A., Kreuzer, M. (1999) Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. *Canadian Journal of Animal Science* 79, 65-72.

Machmüller, A., Ossowski, D.A., Kreuzer, M. (2000) Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. *Animal Feed Science and Technology* 85, 41-60.

Machmüller, A., Soliva, C.R., Kreuzer, M. (2003a) Effect of coconut oil and defaunation treatment on methanogenesis in sheep. *Reproduction Nutrition Development* 43, 41-55.

Machmüller, A., Soliva, C.R., Kreuzer, M. (2003b) Methane-suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion. *British Journal of Nutrition* 90, 529-540.

Martin, C., Rouel, J., Jouany, J.P., Doreau, M., Chilliard, Y. (2008) Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. *Journal of Animal Science* 86, 2642-2650.

McGinn, S.M., Beauchemin, K.A., Coates, T., Colombatto, D. (2004) Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. *Journal of Animal Science* 82, 3346-3356.

Miller, W.H., Koes, R.M. (1988) Construction and operation of an open-circuit indirect calorimetry system for small ruminants. *Journal of Animal Science* 66, 1042-1047.

Mitsumori, M., Ajisaka, N., Tajima, K., Kajikawa, H., Kurihara. M. (2002a) Detection of proteobacteria from the rumen by PCR using methanotroph-specific primers. *Letters in Applied Microbiology* 35, 251-255.

Mitsumori, M., Tajima, K., Itabashi, H. (2002b) Detection of methanogens from the rumen by PCR-based techniques In: Greenhouse Gases and Animal Agriculture (Ed. J. Takahashi and B. A. Young), Elsevier, Amsterdam, 125-128.

Mitsumori, M., Sun, W. (2008) Control of rumen microbial fermentation for mitigating methane emissions from the rumen. *Asian-Australasian Journal of Animal Sciences* 21, 144-154.

Molano, G., Knight, T.W., Clark, H. (2008) Fumaric acid supplements have no effect on methane emissions per unit of feed intake in wether lambs. *Australian Journal of Experimental Agriculture* 48, 165-168.

Newbold, C.J., Lassalas, B., Jouany, J.P. (1995) The importance of methanogens associated with ciliate protozoa in ruminal methane production *in vitro*. *Letters in Applied Microbiology* 21, 230-234.

Odongo, N.E., Or-Rashid, M.M., Kebreab, E., France, J., McBride, B.W. (2007) Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. *Journal of Dairy Science* 90, 1851-1858.

Oliveira, S.G., Berchielli, T.T., Pedreira, M.D.S., Primavesi, O., Frighetto, R., Lima, M.A. (2007) Effect of tannin levels in sorghum silage and concentrate supplementation on apparent digestibility and methane emission in beef cattle. *Animal Feed Science and Technology* 135, 236-248.

O'Mara, F.P. (2011) The significance of livestock as a contributor to global greenhouse gas emissions today and in the near future. *Animal Feed Science and Technology* 166-167, 7-15.

Orskov, E.R., Ryle, M. (1990) Energy nutrition in ruminants. Elsevier Science Publishers Ltd. United Kingdom.

Patra, A.K., Lalhriatpuii, M. (2016) Development of statistical models for prediction of enteric methane emission from goats using nutrient composition and intake variables. Agriculture, Ecosystems and Environment 215, 89-99.

Pinares-Patiño, C.S., Clark, H. (2008) Reliability of the sulfur hexafluoride tracer technique for methane emission measurement from individual animals: an overview. *Australian Journal of Experimental Agriculture* 48, 223-229.

Plaizier, J.C., Krause, D.O., Gozho, G.N., McBride, B.W. 2008 Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. *The Veterinary Journal* 176, 21-31.

Puchala, R., Min, B.R., Goetsch, A.L., Sahlu, T. (2005) The effect of a condensed tannin-containing forage on methane emission by goats. *Journal of Animal Science* 83, 182-186.

Russell, J. B., Wallace, R.J. (1997) Energy-yielding and energy-consuming reactions. In: The rumen microbial ecosystem. 2nd ed. (Ed. Hobson, P.J. and Stewart, C.S.), Blackie Acad. Profess. London, 246-282.

Sauvant D., Giger-Reverdin, S. (2007) Empirical modelling meta-analysis of digestive interactions and CH_4 production in ruminants. In: Energy and protein metabolism and nutrition (Eds. Ortigues-Marty, I., Miraux, N. and Brand-Williams, W.), Wageningen Academic, Wageningen, The Netherlands, 561-563.

Soliva, C.L., Meile, L., Cieślak, A., Kreuzer, M., Machmüller, A. (2004) Rumen simulation technique study on the interactions of dietary lauric and myristic acid supplementation in suppressing ruminal methanogenesis. *British Journal of Nutrition* 92, 689-700.

Storm, I.M.L.D., Hellwing, A.L.F., Nielsen, N.I., Madsen, J. (2012) Methods for measuring and estimating methane emission from ruminants. *Animals* 2, 160-183. doi:10.3390/ani2020160

Takahashi, J., Chaudhry, A.S., Beneke, R.G., Young, B.A. (1999) An open-circuit hood system for gaseous exchange measurements in small ruminants. *Small Ruminant Research* 32, 31-36. doi:10.1016/S0921-4488(98)00163-1

Tavendale, M.H., Meagher, L.P., Pacheco, D., Walker, N., Attwood, G.T., Sivakumaran, S. (2005) Methane production from *in vitro* rumen incubations with Lotus pedunculatus and Medicago sativa, and effects of extractable condensed tannin fractions on methanogenesis. *Animal Feed Science and Technology* 123-124, 403-419.

Tiemann, T.T., Lascano, C.E., Wettstein, H.R., Mayer, A.C., Kreuzer, M., Hess, H.D. (2008) Effect of the tropical tannin-rich shrub legumes *Calliandra calothyrsus* and *Flemingia macrophylla* on methane emission and nitrogen and energy balance in growing lambs. *Animal* 2, 790-799.

Ueno, Y., Yamada, K., Yoshida, N., Maruyama, S., Isozaki, Y. (2006) Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature* 440, 516-519.

Ulyatt, M.J., McCrabb, G.J., Baker, S.K., Lassey. K.R. (1999) Accuracy of SF_6 tracer technology and alternatives for field measurements. *Australian Journal of Agricultural Research* 50, 1329-1334.

Van Kessel, J.A.S., Russell, J.B. (1996) The effect of pH on ruminal methanogenesis. *FEMS Microbiology Ecology* 20, 205-210.

Vlaming, J.B., Brookes, I.M., Hoskin, S.O., Pinares-Patiño, C. S., Clark, H. (2007) The possible influence of intra-ruminal sulphur hexafluoride release rates on calculated methane emissions from cattle. *Canadian Journal of Animal Science* 87, 269-275.

Wilkerson, V.A., Casper, D.P., Mertens, D.R. (1995) The prediction of methane production of Holstein cows by several equations. *Journal of Dairy Science* 78, 2402-2414.

Wolin, M. J., Miller, T.L., Stewart, C.S. (1997) Microbemicrobe interactions In: The rumen microbial ecosystem. 2nd ed. (Ed. Hobson, P.J. and Stewart, C.S.), Blackie Acad. Profess. London, 467-491.

Wood, T.A., Wallace, R.J., Rowe, A., Price, J., Yáñez-Ruiz, D.R., Murray, P., Newbold, C.J. (2009) Encapsulated fumaric acid as a feed ingredient to decrease ruminal methane emissions. *Animal Feed Science and Technology* 152, 62-71. doi:10.1016/j.anifeedsci.2009.03.006

Wright, A.D.G., Kennedy, P., O'Neill, C.J., Toovey, A.F., Popovski, S., Rea, S.M., Pimm, C.L., Klein, L. (2004) Reducing methane emissions in sheep by immunization against rumen methanogens. *Vaccine* 22, 3976-3985.